BASF
The Chemical Company

DOSSIER FOR THE EVALUATION OF THE ACTIVE SUBSTANCE

Bentazone (BAS 351 H)

Data requirement according to Regulation (EU) No. 544/2011 and (EU) No. 545/2011 (formerly Annex II and III of Directive 91/414/EEC) using OECD Guidance for Industry Data Submissions on Plant Protection Products and their Active Substance
(Dossier Guidance)

Document M-II

Summary and evaluation (Tier II)

Section 1

Identity, physical and chemical properties and further information

BASF DocID 2012/1007331

compiled by

[redacted]

Date: 24 February 2012

1 Identity of the active substance

List of Contents

According to Article 1(c) of Regulation (EU) No. 1141/2010 the supplementary dossier includes data and risk assessments which were not part of the original dossier and which are necessary to reflect changes. Reasoning for each test or study as required by Art 1(d)/(e) of the Regulation is given in the reference list of the dossier.

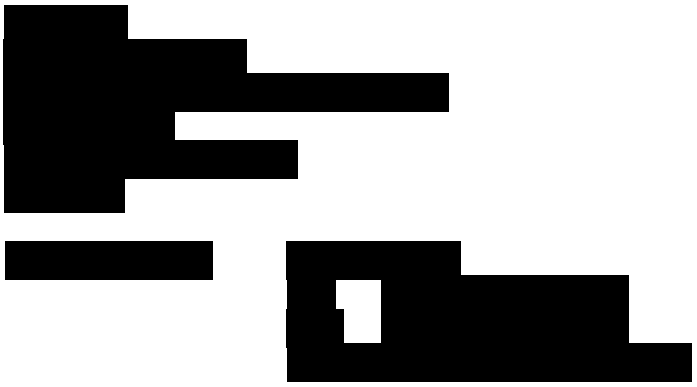
1.1 Applicant (name, address, contact, phone and fax numbers)

1.1.1 Headquarter/Germany

BASF SE
D-67056 Ludwigshafen
Germany

BASF SE
Agricultural Center
Product Registration Management
Postfach 120
D-67114 Limburgerhof
Germany

1.1.2 Affiliates or representatives



1.1.3 Alternate

1.2 Manufacturer(s) (name, address, contact, phone and fax numbers)

[redacted]

This confidential information is provided in Document JM2.

1.3 ISO common name proposed or accepted, and synonyms

Common name (ISO): bentazone

When this substance is used as a salt, its identity is stated as bentazone sodium. The name “bendioxide” is used in South Africa, and the name “bentazon” is used in Canada and the USA.

1.4 Chemical name

1.4.1 IUPAC nomenclature

IUPAC name: 3-isopropyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide

1.4.2 CA nomenclature

CA nomenclature: 3-(1-methylethyl)-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide

1.4.3 Alternative nomenclature

not assigned

1.5 Manufacturer's codes, names and patent status

1.5.1 Manufacturer's code number(s), incl. countries and periods where used

Bentazone has the following codes:

BASF Registry Number:	Reg.No. 51929
BAS-No.:	BAS 351 H

The BASF Registry Number for Bentazone sodium salt is Reg.No. 88691

1.5.2 Trade name(s)

Information not required for registration in EU.

1.5.3 Patent status

Information not required for registration in EU.

1.6 Existing CAS, CIPAC, EINECS and ELINCS numbers

	Bentazone	Bentazone sodium
CAS No.	25057-89-0	50723-80-3
EINECS No.	246-585-8	256-735-4
CIPAC No.	366	not assigned
ELINCS No.	not assigned	not assigned

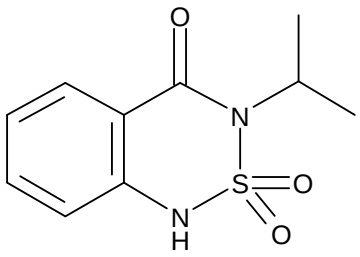
1.7 Molecular formula, molecular mass and structural formula

Bentazone:

Molecular formula: $C_{10}H_{12}N_2O_3S$

Molecular Mass: 240.3 g/mol

Structural formula:

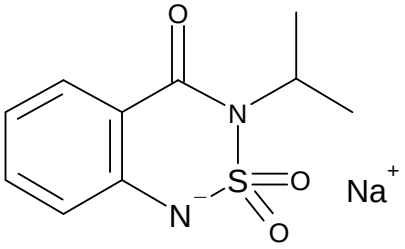


Bentazone sodium

Molecular formula: $C_{10}H_{11}N_2NaO_3S$

Molecular Mass: 262.3 g/mol

Structural formula:



1.8 Method of manufacture

1.8.1 Method of manufacture for each plant

Confidential information provided in Doc JM2.

1.8.2 Description of starting materials

Confidential information provided in Doc JM2.

1.9 Specification of purity of the active substance

1.9.1 Minimum and/or nominal content (g/kg) of pure active substance

The minimum purity of 960 g/kg related to the theoretically dry active ingredient. Typically bentazone is handled as technical concentrate with ca. 700 g/L bentazone sodium salt (corresponding to 640 g/L bentazone) in water.

1.9.2 Certified limits of the active substance

This confidential information is given in Document JM 2.

1.9.3 Control product specification form/confidential statement of formula

This confidential information is given in Document JM 2.

1.10 Identity, content and structure of isomers, impurities and additives

1.10.1 Inactive isomers

This confidential information is given in Document JM 2.

1.10.2 Impurities and additives

This confidential information is given in Document JM 2.

1.11 Batch analysis data

1.11.1 Analytical profile of batches

This confidential information is given in Document JM 2.

1.11.2 Results of analyses of batches used in toxicological testing

This confidential information is given in Document JM 2.

1.12 Other/special studies

There are no other/special studies.

According to Article 1(c) of Regulation (EU) No. 1141/2010 the supplementary dossier includes data and risk assessments which were not part of the original dossier and which are necessary to reflect changes. Reasoning for each test or study as required by Art 1(d)/(e) of the Regulation is given in the reference list of the dossier.

2 Physical and chemical properties of the active substance

Remark: in order to have a complete picture of the physico-chemical properties of bentazone active ingredient technical grade and pure all results are listed in the table below. Basically all those results deriving from reports dated prior to 1999 were submitted in the previous EU-authorization, and reviewed and summarized in the EU Monograph.
The already assessed end-points are highlighted in italics and new data are highlighted in bold, so that it is obvious, which data have been prepared additionally within the recent years. Data that were submitted in the previous EU-authorization, but are no longer considered valid are listed by Annex point in the list of studies not submitted.

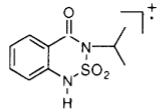
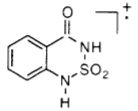
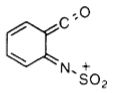
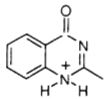
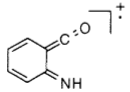
Annex Point IIA	Guideline and method	Test material purity and Specification	Findings	Comments	GLP Y/N	Author, Year
Melting point and boiling point (2.1)						
Melting point, freezing point or solidification point, purified a.s. (2.1.1)	OECD 102 DSC	01893-210: 99.8%	**Melting point: 139 °C (onset temperature)**	In accordance with the endpoint: of initial submission from 1994: 139.4- 140.1 °C Repetition necessary due to lack of DSC curve.	Y	2011/1074521 Kroehl T. 2011d
	OECD 102 DSC	Bentazone sodium COD-001417: 91.9%	**Decomposes prior to melting**		Y	2011/1074522 Kroehl T. 2011e

Annex Point IIA	Guideline and method	Test material purity and Specification	Findings	Comments	GLP Y/N	Author, Year
Boiling point of purified active substance (2.1.2)			*No boiling or sublimation of bentazone PAI up to 210 °C decomposition temperature*			
Temperature at which decomposition or sublimation occurs (2.1.3)	OECD 102 DSC	01893-210: 99.8%	**Decomposition temperature: 157 °C (onset temperature)**	Results of initial submission in 1994: decomposition at ca. 210 °C (gas evolution)	Y	2011/1074521 Kroehl T. 2011d
	OECD 102 DSC	COD-001417: 91.9%	**Decomposition temperature: 212 °C (begin)**		Y	2011/1074522 Kroehl T. 2011e
Relative density of purified active substance (2.2)	EEC A 3.1.4.3	WH 11932, Bentazon: 99.8%	*D_4^{20}.: 1.405 at 20°C*		Y	1994/10783 Kroehl T. 1994a
Vapour pressure and volatility (2.3)						
Vapour pressure of purified active substance (2.3.1)	EEC A4	Bentazone 01196-1: 99.6%	4.9*10-4Pa at 20°C		Y	1999/11055 Kaestel R. 1999c
Henry's law constant (2.3.2)		not relevant	**At 20 °C: 2.108* 10 $^{-9}$ kPa.m³. mol^{-1}**		N	2000/1023935 Brem G. 2000a
Appearance (2.4)						
Description of the physical state and colour, pure and techn. a.s.	Visual assessment	Bentazone, Batch 691-16-1 99.8 %	*Bentazone PAI is a solid consisting of white crystals.*		Y	1994/ 11115 Tuerk W. 1994c

Annex Point IIA	Guideline and method	Test material purity and Specification	Findings	Comments	GLP Y/N	Author, Year
(2.4.1)	Visual assessment	N 194, WH 11092, Bentazon: 97.6%	*Bentazone TC is a solid consisting of a yellow powder.*		Y	1993/11290 Kaestel R. 1993a
	Visual assessment	COD-001417: 91.9%	**Bentazone Na is a light yellow powder.**		Y	2011/1074522 Kroehl T. 2011e
	Visual assessment	B135-1/9: 644.9 g/L	**Bentazone Na salt technical concentrate is a red-brown clear liquid.**		Y	2011/1074527 Kroehl T. 2011f
Description of the odour - purified and technical active substance (2.4.2)	Organoleptic assessment	691-16-1: 99.8%	*Bentazone PAI is odourless*		Y	1994/11193 Tuerk W. 1994a
	Organoleptic assessment	N 194, WH 11092, Bentazon: 97.6%	*Bentazone TGAI is odourless*		Y	1993/11290 Kaestel R. 1993a
	Organoleptic assessment	COD-001417: 91.9%	**Bentazone Na is odourless..**		Y	2011/1074522 Kroehl T. 2011e
	Organoleptic assessment	B135-1/9: 644.9 g/L	**Bentazone Na salt technical concentrate is odourless.**		Y	2011/1074527 Kroehl T. 2011f
Spectra and molecular extinction at relevant wavelengths (2.5)						
Spectra for purified active substance (2.5.1)		**01893-210: 99.8%**	**The spectra confirm the identity of the proposed structure.**		**Y**	**2011/1074523 Kroehl T. 2011c**

<table>
<tr><th>Annex Point IIA</th><th>Guideline and method</th><th>Test material purity and Specification</th><th>Findings</th><th>Comments</th><th>GLP Y/N</th><th>Author, Year</th></tr>
<tr><td>UV/VIS
(2.5.1.1)</td><td></td><td></td><td>Bentazone in methanol (pH4.2):
<table>
<tr><th>Wavelength Lamda</th><th>Absorption Units</th><th>ε [l x mol^{-1} x cm^{-1}]</th></tr>
<tr><td>215</td><td>1.073</td><td>25938</td></tr>
<tr><td>227</td><td>0.6661</td><td>16102</td></tr>
<tr><td>242</td><td>0.3395</td><td>8207</td></tr>
<tr><td>290</td><td>0,0406</td><td>981</td></tr>
<tr><td>311</td><td>0.0573</td><td>1385</td></tr>
<tr><td>340</td><td>0.0452</td><td>1093</td></tr>
</table>
Bentazone in water (pH 4.4):
<table>
<tr><th>Wavelength Lamda</th><th>Absorption Units</th><th>ε [l x mol^{-1} x cm^{-1}]</th></tr>
<tr><td>224</td><td>1.3314</td><td>32184</td></tr>
<tr><td>246</td><td>0.3356</td><td>8113</td></tr>
<tr><td>290</td><td>0.0104</td><td>251</td></tr>
<tr><td>336</td><td>0.1298</td><td>3138</td></tr>
</table>
Bentazone under acidic conditions (pH 1.4):
<table>
<tr><th>Wavelength Lamda</th><th>Absorption Units</th><th>ε [l x mol^{-1} x cm^{-1}]</th></tr>
<tr><td>213</td><td>1.1885</td><td>28730</td></tr>
<tr><td>242</td><td>0.3841</td><td>9285</td></tr>
<tr><td>290</td><td>0.0712</td><td>1721</td></tr>
<tr><td>302</td><td>0.0848</td><td>2050</td></tr>
</table>
Bentazone under basic conditions (pH 12.3)
<table>
<tr><th>Wavelength Lamda</th><th>Absorption Units</th><th>ε [l x mol^{-1} x cm^{-1}]</th></tr>
<tr><td>224</td><td>1.3824</td><td>33417</td></tr>
<tr><td>246</td><td>0.3393</td><td>8202</td></tr>
<tr><td>290</td><td>0.0141</td><td>341</td></tr>
<tr><td>335</td><td>0,1361</td><td>3290</td></tr>
</table></td><td></td><td></td><td></td></tr>
</table>

Annex Point IIA	Guideline and method	Test material purity and Specification	Findings	Comments	GLP Y/N	Author, Year
IR (2.5.1.2)		691/16-1: 99.8%	3118.0 cm^{-1} (ν N-H) 1657.8 cm^{-1} (ν C=O) 1479.2 cm^{-1} (ν C=C, aromatic) 1420.3 cm^{-1} (δ C-H, Methyl) 1349.7 / 1171.7 cm^{-1} (ν S=O in - SO_2) 761.3 cm^{-1} (δ C-H out-of-plane, ortho disubstituted aromatic ring)		Y	1994/11112 Tuerk W. 1994b
NMR (2.5.1.3)		691/16-1: 99.8%	1H-NMR: 1.58 ppm (d,6H,H9+H10) 5.02 ppm (m,1H,H8) 7.09 ppm (dd,1H,H6) 7.34 ppm (dt,1H,H5) 7.57 ppm (dt,1H,H4) 7.70 ppm (s,1H,H11) 8.15 ppm (dd,1H,H3)		Y	1994/11112 Tuerk W. 1994b
		691-16-2: 99.8%	13C-NMR: 20.6 ppm (C8,C10) 49.3 ppm (C9) 120.25 ppm (C5) 120.35 ppm (C1) 125.7 ppm (C3) 130.3 ppm (C2) 134.8 ppm (C4) 136.2 ppm (C6) 162.6 ppm (C7)		Y	2000/1018683 Daum A. 2000d

Annex Point IIA	Guideline and method	Test material purity and Specification	Findings	Comments	GLP Y/N	Author, Year
MS (2.5.1.4)		691/16-1: 99.8%	m/z 240  m/z 225 M - CH_3 m/z 198  m/z 182  m/z 161  m/z 120 161 - CH_3CN m/z 119  m/z 92 120 - CO, 119 - HCN m/z 64 		Y	1994/11112 Tuerk W. 1994b
Wavelengths at which UV/VIS molecular extinction occurs, max > 290 nm (2.5.1.5)		691/16-1: 99.8%	Results see above.		Y	1994/11112 Tuerk W. 1994b
optical purity (2.5.1.6)			Bentazone is not optically active.			
Spectra for impurities (2.5.2)		not relevant	see JM2 for information on impurities.			
UV/VIS (2.5.2.1)						
IR (2.5.2.2)						

Annex Point IIA	Guideline and method	Test material purity and Specification	Findings	Comments	GLP Y/N	Author, Year
NMR (2.5.2.3)						
MS (2.5.2.4)						
Solubility of purified active substance in water (pH 4-10) (2.6)		01893-210: 99.8%	**Bentazone (BAS 351 H, Reg. No. 051929)** Solubility in Water at 20 °C by Flask Method — g/L H_2O (demin) pH 7: Average 0.57; Standard Dev. 0.02; Actual pH at equilibrium 3.5 - 4 pH 7 Phosphate Buffer: Average 7.7; Standard Dev. 0.2; Actual pH at equilibrium 4 pH 4 Citrate Buffer: Average 3.0; Standard Dev. 0.2; Actual pH at equilibrium 3.5 pH 9 Borate Buffer: Average 17; Standard Dev. 0.3; Actual pH at equilibrium 4		Y	2011/1074524 Class T. 2001a
Solubility in organic solvents at 15 to 25°C (2.7)	CIPAC MT 181 OECD 105	N 187: 96.9%	Solubility in toluene: 24.5 g/L (± 1.6 g/L) Solubility in heptane: 0.11 g/L (± 0.1 g/L)	These results confirm the originally submitted results: poor solubility in n-heptane (0.05 g/L), low solubility in toluene (21 g/L) good solubility in dichloromethane, methanol, acetone and ethyl actetate (between ca. 170 to 670 g/L)	Y	2011/1074525 Class T. 2011c
Partition coefficient (2.8)						
n-Octanol/water partition coefficient (2.8.1)	EEC A.8 OECD 107	01196-1: 99.6%	deionized water: log pOW: 1.49 pOW:30.6 buffer pH 4: log pOW: 1.54 pOW: 35.2 buffer pH7: log pOW: -0.94 pOW:0.12 buffer pH9: log pOW: -1.32 pOW:0.05	Bentazone does not bioaccumulate	Y	2000/1018475 Daum A. 2000e

Annex Point IIA	Guideline and method	Test material purity and Specification	Findings	Comments	GLP Y/N	Author, Year
Effect of pH (4 to 10) on the n-octanol/water partition coefficient (2.8.2)	EEC A.8 OECD 107	01196-1: 99.6%	see above.		Y	2000/1018475 Daum A. 2000e
Hydrolysis and photolysis (2.9)						
Hydrolysis rate at pH 4, 7 and 9 under sterile and dark conditions (2.9.1)	*US-EPA-161-1*		*At 25 °C bentazone is hydrolytically stable at pH 5, 7 and 9.*		*Y*	*1986/5018 Panek E. 1986a*
Direct phototransformation in sterile water using artificial light (2.9.2)	**OECD 316 US-EPA OPPTS 835.22140**	**210/12201 (phenyl-U-14C): purity 97.3%, specific activity 317400 dpm/µg**	**DT50 parent < 5.5 d (tested at pH 5, 7, and 9) metabolites = Peak B 27%, 18%, 9% at pH 5, 7, 9 respectively Peak C 20%, 25%, 23% at pH 5, 7, 9 respectively all others <= 6%**		Y	**2011/7002318 Singh M. 2011a**
Quantum yield of direct phototransformation (2.9.3)	**OECD 316 US-EPA OPPTS 835.22140**	**210/12201 (phenyl-U-14C): purity 97.3%, specific activity 317400 dpm/µg**	**Quantum Yield = 7.7 * 10^{-3} mol/Einstein in aqueous photolysis at pH 5**		Y	**2011/7002318 Singh M. 2011a**

Annex Point IIA	Guideline and method	Test material purity and Specification	Findings	Comments	GLP Y/N	Author, Year
Lifetime in the top layer of aqueous systems (calculated and real) (2.9.4)	**OECD 316 US-EPA OPPTS 835.2240**	**210/12201 (phenyl-U-14C): purity 97.3%, specific activity 317400 dpm/µg**	experimental DT50 pH 5 3.3 days (r^2 = 0.977) pH 7 5.4 days (r^2 = 0.965) pH 9 3.9 days (r^2 = 0.968) calculated: pH 5 1.2 h (June) - 11.1 h (Dec.) pH 7 2.0 h (June) - 18.2 h (Dec.) pH 9 1.4 h (June) - 13.3 h (Dec.)		Y	**2011/7002318 Singh M. 2011a**
Dissociation in water of purified active substance (2.9.5)	OECD 112, titration method	01196-1: 99.6%	The dissociation constant was determined to be 3.51		Y	2000/1013485 Daum A. 2000f
		not relevant	The partition coefficient of the non-ionized form of bentazone was calculated from the pka for the pH range from pH 1-6. The calculations show, that bentazone is completely nonionized at pH values below 1.2 and completely ionized at pH values above 5.8.		N	2002/1007049 Daum A. 2002b
Estimated photochemical oxidative degradation (2.10)	*Calculated according to Atkinson*	not relevant	*Constant for reaction with OH-radicals:* *Atmospheric degradation half-life:* *$T_{1/2} \leq$ 2.1 h*		*N*	EU review report von Bentazon 7585/VI/97-final, Nov. 2000
Flammability including auto-flammability (2.11)						
Flammability of the active substance as manufactured (2.11.1)			Not required for liquids			

Annex Point IIA	Guideline and method	Test material purity and Specification	Findings	Comments	GLP Y/N	Author, Year
Auto-flammability of the active substance as manufactured (2.11.2)	**EEC A.9**	**Technical concentrate: B 135-1/9: 644.9 g/L**	**Auto ignition temperature: 537 °C**		Y	**2011/1074526 Gundrum C. 2012a**
Flash point of the active substance as manufactured (2.12)	**EEC A.9**	**Technical concentrate: B 135-1/9: 644.9 g/L**	**No flashpoint.**		Y	**2011/1074526 Gundrum C. 2012a**
Explosive properties of the active substance as manufactured (2.13)	**OECD 113** **EEC A.14**	**Technical concentrate: B 135-1/9: 644.9 g/L**	**OECD 113: in the temperature range 30-500°C 1 reaction:** **onset 170°C, peak temperature: 206°C; energy release: 360 J/g.** **The test A.14 has not been carried out. Reason: The energy release in test OECD 113 was less than 500 J/g**		Y	**2011/1074526 Gundrum C. 2012a**
Surface tension of the active substance as manufactured (2.14)	*OECD 115, Ring method*	*Bentazone PAI: 99.8 % WH 11932*	*Surface tension, concentration (w/w) in pure water:* *0.5 %: 69.2 mN/m* *2.0 %: 70.0 mN/m*		*Y*	*1994/10783 Kroehl 1994a*
Oxidizing properties of the active substance as manufactured (2.15)	**EEC A.21**	**Technical concentrate: B 135-1/9: 644.9 g/L**	**Not oxidizing.**		Y	**2011/1074526 Gundrum C. 2012a**
pH (2.16)	**CIPAC MT 75**	WH 7211 600g/L	pH at 0.2 % concentration in pure water: 6.4 pH at 6.7 % concentration in pure water: 7.4		Y	**1990/10080 Gueckel W. 1990a**
	CIPAC MT 75	**B135-1/9: 644.9 g/L**	**pH neat technical concentrate: 9.2** **pH at 1% concentration in CIPAC water D: 7.8** **pH at 1 % concentration in pure water: 7.6**		Y	**2011/1074527 Kroehl T. 2011f**

Annex Point IIA	Guideline and method	Test material purity and Specification	Findings	Comments	GLP Y/N	Author, Year
Stability (2.17)						
Storage stability (2.17.1)	**Storage for 18 year at ambient conditions**	**Bentazon dry TGAI N 187: 96.9%**	**Initital ananlysis of batch 187 (1986): 97.8 %. Result after 18 years storage under ambient conditions : 96.9 % Bentazone is stable under ambient conditions.**	**see also JM2 for confidential information on tox batches**	Y	**2004/1038794 Genari G. 2004**
Stability (temperature, metals) (2.17.2)			Not required.			
Other/special studies (2.18)			None deemed necessary.			

Summary and discussion

Bentazone, chemical name (IUPAC 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide) is a herbicide and belongs to the chemical group of benzothiadiazinones. It had been assessed during an EU process within the time of 1995 to 2000 and listed in Annex I in 2000. Many data concerning the physico chemical properties had already been provided and assessed during this first evaluation. However, as scientific and methodical approach to the data improved during the time, besides the already assessed values, the new established endpoints are listed in the table above. The data that have not changed within the time interval of nearly 15 years are in italics and new data highlighted in bold. Two major differences between the data from 2000 and those of 2012 are the addition of data on the technical concentrate (TK) and on the sodium salt of bentazone. The 640 g/L bentazone TK is the manufactured product. Formerly, mainly the free bentazone (produced by an additional precipitation step from the technical concentrate) had been described.

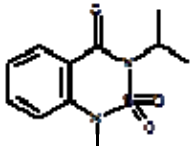
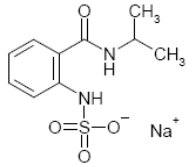
The pure active ingredient bentazone consists of white powder, bentazone sodium is a slightly yellow powder. The technical concentrate is a red liquid. All samples investigated were odourless. In water with pH values above 6.8 bentazone is completely dissociated. It has only low potential for bioaccumulation in the environment (log pOW: 1.54 at pH 4). The vapor pressure and the volatility of bentazone are low. The solubility in organic solvents ranges from poor (n-heptane: 0.05 g/L) to excellent (ethyl acetate 670 g/L).The solubility of bentazone in pure water is low (0.57 g/L), however by adding buffer and with increasing pH the solubility increases to 30 g/L at pH 9. Bentazone sodium is completely soluble in water. Bentazone TK is not highly flammable, not explosive or oxidizing. Bentazone decreases by photolysis (DT_{50} < 5.5 d) and is hydrolytically stable.

The active ingredient is stable under warehouse conditions over many years.

Physical and chemical properties of metabolites

Depending on pH and temperature, bentazone can be degraded to several cleavage products. The analytical results for certain physico-chemical characteristics of two potential metabolites are summarized in table 2.2. Additionally, the degradates are identified with respect to molecular structure, chemical name, molecular mass and BASF internal Reg. No. Further details on these metabolites are discussed in the respective dossier parts dealing with their formation.

Table 2.2: Physico-chemical properties of bentazone metabolites

Metabolite Structural formula	Description		Chemical and physical data	Guideline and method	Purity	Findings and comments	GLP Y/N	Reference
	BASF Reg.No.: Other internal code(s): CAS registry number: Molecular formula: Molecular mass: Chemical name:	79520 BH 351-N-Me 61592-45-8 C_{11} H_{14} N_2O_3 S 254.31 g/mol 3-isopropyl-1-methyl-2,1,3-benzothia-diazin-4(3*H*)-one 2,2-dioxide	Water solubility (2.6)	OECD 105 EEC A.6 Flask Method	L45-197: 99.7%	The solubility in pure water at 20°C was determined to be 74 mg/l. The pH of the saturated solution was 6.7.	Y	2004/1006562 Daum A., 2004
			Octanol/water-partition coefficient (2.8)	OECD 117 EEC A.8	L45-197: 99.7%	log Pow = 2.65 in two different eluents at 20°C	Y	2003/1018554 Daum A. 2003
	BASF Reg.No. Other internal code (s): CAS registry number: Molecular formula: Molecular mass: Chemical name::	5051517 M351H024 -/- C_{10} H_{13} N_2Na O_4 S 280,28 g/mol sodium 2-[(isopropyl-amino)-carbonyl] phenylsulfamate	Water solubility (2.6)	OECD 105	L84-56: 96.0%	Completely soluble (> 1 kg/L) in water at pH 4, pH 7 and pH 9.	Y	2012/1012904 Class, 2012

3 Further Information on the active substance

List of Contents

3.1 Function e.g. fungicide

Efficacy documentation is provided in Annex III chapter 6.

In accordance with the “Guidance Document on the renewal of active substances included in Annex I of Council Directive 91/414/EEC to be assessed in compliance with Regulation 1141/2010 (the renewal regulation)“ (SANCO10387/2010 rev. 8) no other efficacy documentation is deemed to be necessary.

3.2 Effects on harmful organisms e.g. contact action

3.2.1 Nature of the effects on harmful organisms

See chapter 3.1

3.2.2 Translocation in plants

See chapter 3.1

3.3 Fields of use e.g. forestry

See chapter 3.1

3.4 Harmful organisms controlled and crops / products protected or treated

3.4.1 Details of existing and intended uses

See chapter 3.1

3.4.2 Details of harmful organisms against which protection is afforded

See chapter 3.1

3.4.3 Effects achieved e.g. sprout suppression

See chapter 3.1

3.5 Mode of action

3.5.1 Mode of action, mechanism(s) and pathway(s) involved

See chapter 3.1

3.5.2 Details of active metabolites and degradation products

See chapter 3.1

3.5.3 Formation of active metabolites and degradation products

See chapter 3.1

3.6 Possible development of resistance or cross-resistance

See chapter 3.1

3.7 A material safety data sheet for the active substance

Report: II A 3.7/1
Anonymous 2012(c)
Safety data sheet - Bentazon NA tech.
BASF DocID 2012/1056646
Guidelines: EEC 1907/2006
GLP: No, not subject to GLP regulations

1. Identification of the substance/mixture and of the company/undertaking

Product identifier

BENTAZON NA TECHN.

Relevant identified uses of the substance or mixture and uses advised against

Relevant identified uses: crop protection active ingredient

Details of the supplier of the safety data sheet

Company:
BASF SE
67056 Ludwigshafen
GERMANY
Operating Division Crop Protection

Telephone: +49 621 60-27777
E-mail address: Produktinformation-Pflanzenschutz@basf.com

Emergency telephone number

International emergency number:
Telephone: +49 180 2273-112

2. Hazards Identification

Label elements

Globally Harmonized System, EU (GHS)

Pictogram:

Signal Word:
Warning

Hazard Statement:

H319	Causes serious eye irritation.
H302	Harmful if swallowed.
H317	May cause an allergic skin reaction.
H412	Harmful to aquatic life with long lasting effects.

Precautionary Statements (Prevention):

P280f	Wear protective gloves and eye/face protection.
P261c	Avoid breathing mist.
P264	Wash with plenty of water and soap thoroughly after handling.
P270	Do no eat, drink or smoke when using this product.
P272	Contaminated work clothing should not be allowed out of the workplace.

Precautionary Statements (Response):

P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P363	Wash contaminated clothing before reuse.
P301 + P312	IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P330	Rinse mouth.

Precautionary Statements (Disposal):

P501	Dispose of contents/container to hazardous or special waste collection point.

According to Regulation (EC) No 1272/2008 [CLP]

Hazard determining component(s) for labelling: BENTAZONE-SODIUM

According to Directive 67/548/EEC or 1999/45/EC

EEC Directives

Hazard symbol(s)
Xn Harmful.

R-phrase(s)

R22	Harmful if swallowed.
R43	May cause sensitization by skin contact.
R52/53	Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

S-phrase(s)

S24	Avoid contact with skin.
S37	Wear suitable gloves.

Hazard determining component(s) for labelling: BENTAZONE-SODIUM

Classification of the substance or mixture

According to Regulation (EC) No 1272/2008 [CLP]

Acute Tox. 4 (oral)
Eye Dam./Irrit. 2
Skin Sens. 1
Aquatic Chronic 3

According to Directive 67/548/EEC or 1999/45/EC

Possible Hazards:
Harmful if swallowed.
May cause sensitization by skin contact.
Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

For the classifications not written out in full in this section the full text can be found in section 16.

Other hazards

According to Regulation (EC) No 1272/2008 [CLP]

See section 12 - Results of PBT and vPvB assessment.

If applicable information is provided in this section on other hazards which do not result in classification but which may contribute to the overall hazards of the substance or mixture.

3. Composition/Information on Ingredients

Mixtures

Chemical nature

technical preconcentrate

Contains: bentazone
as Bentazone Sodium salt

Hazardous ingredients (GHS)
according to Regulation (EC) No. 1272/2008

bentazone-sodium

Content (W/W): 55.9 %	Acute Tox. 4 (oral)
CAS Number: 50723-80-3	Skin Sens. 1
EC-Number: 256-735-4	H302, H317

Hazardous ingredients
according to Directive 1999/45/EC

bentazone-sodium

Content (W/W): 55.9 %
CAS Number: 50723-80-3
EC-Number: 256-735-4
Hazard symbol(s): Xn
R-phrase(s): 22, 43

For the classifications not written out in full in this section, including the indication of danger, the hazard symbols, the R phrases, and the hazard statements, the full text is listed in section 16.

4. First-Aid Measures

Description of first aid measures

Remove contaminated clothing.

If inhaled:
Keep patient calm, remove to fresh air, seek medical attention.

On skin contact:
Wash thoroughly with soap and water.

On contact with eyes:
Wash affected eyes for at least 15 minutes under running water with eyelids held open, consult an eye specialist.

On ingestion:
Immediately rinse mouth and then drink 200-300 ml of water, seek medical attention.

Most important symptoms and effects, both acute and delayed
Symptoms: The most important known symptoms and effects are described in the labelling (see section 2) and/or in section 11., Further important symptoms and effects are so far not known.

Indication of any immediate medical attention and special treatment needed
Treatment: Treat according to symptoms (decontamination, vital functions), no known specific antidote.

5. Fire-Fighting Measures

Extinguishing media
Suitable extinguishing media:
water spray, foam, dry powder, carbon dioxide

Special hazards arising from the substance or mixture
carbon monoxide, Carbon dioxide, nitrogen oxides, sulfur oxides
The substances/groups of substances mentioned can be released in case of fire.

Advice for fire-fighters
Special protective equipment:
Wear self-contained breathing apparatus and chemical-protective clothing.

Further information:
Collect contaminated extinguishing water separately, do not allow to reach sewage or effluent systems. Dispose of fire debris and contaminated extinguishing water in accordance with official regulations. In case of fire and/or explosion do not breathe fumes. Keep containers cool by spraying with water if exposed to fire.

6. Accidental Release Measures

Personal precautions, protective equipment and emergency procedures
Do not breathe vapour/spray. Use personal protective clothing. Avoid contact with the skin, eyes and clothing.

Environmental precautions
Do not discharge into drains/surface waters/groundwater. Do not discharge into the subsoil/soil.

Methods and material for containment and cleaning up
For small amounts: Pick up with suitable absorbent material (e.g. sand, sawdust, general-purpose binder, kieselguhr).
For large amounts: Dike spillage. Pump off product.
Dispose of absorbed material in accordance with regulations. Collect waste in suitable containers, which can be labeled and sealed. Clean contaminated floors and objects thoroughly with water and detergents, observing environmental regulations.

Reference to other sections
Information regarding exposure controls/personal protection and disposal considerations can be found in section 8 and 13.

7. Handling and Storage

Precautions for safe handling

No special measures necessary if stored and handled correctly. Ensure thorough ventilation of stores and work areas. When using do not eat, drink or smoke. Hands and/or face should be washed before breaks and at the end of the shift.

Protection against fire and explosion:
No special precautions necessary. The substance/product is non-combustible. Product is not explosive.

Conditions for safe storage, including any incompatibilities

Segregate from foods and animal feeds.
Further information on storage conditions: Keep away from heat. Protect from direct sunlight.

Storage stability:
Storage duration: 60 Months

Protect from temperatures below: 0 °C
Changes in the properties of the product may occur if substance/product is stored below indicated temperature for extended periods of time.
Protect from temperatures above: 40 °C
Changes in the properties of the product may occur if substance/product is stored above indicated temperature for extended periods of time.

Specific end use(s)

For the relevant identified use(s) listed in Section 1 the advice mentioned in this section 7 is to be observed.

8. Exposure Controls/Personal Protection

Control parameters

Components with workplace control parameters

50723-80-3: 3-Isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide, sodium salt

Exposure controls

Personal protective equipment

Respiratory protection:
Suitable respiratory protection for higher concentrations or long-term effect: Combination filter for gases/vapours of organic, inorganic, acid inorganic and alkaline compounds (e.g. EN 14387 Type ABEK).

Hand protection:
Suitable chemical resistant safety gloves (EN 374) also with prolonged, direct contact (Recommended: Protective index 6, corresponding > 480 minutes of permeation time according to EN 374): E.g. nitrile rubber (0.4 mm), chloroprene rubber (0.5 mm), butyl rubber (0.7 mm) and other

Eye protection:
Safety glasses with side-shields (frame goggles) (e.g. EN 166)

Body protection:
Body protection must be chosen depending on activity and possible exposure, e.g. apron, protecting boots, chemical-protection suit (according to EN 14605 in case of splashes or EN ISO 13982 in case of dust).

General safety and hygiene measures
Handle in accordance with good industrial hygiene and safety practice. Wearing of closed work clothing is recommended. Store work clothing separately. Keep away from food, drink and animal feeding stuffs.

9. Physical and Chemical Properties

Information on basic physical and chemical properties

Form:	liquid	
Colour:	red-brown	
Odour:	odourless	
Odour threshold:	not determined	
pH value:	approx. 8 - 9 (CIPAC standard water D, 1 %(m), 20 °C)	
Melting point:	approx. 0 °C (1,013.3 hPa) Information applies to the solvent.	
Boiling point:	approx. 100 °C (1,013.3 hPa) Information applies to the solvent.	
Flash point:	Non-flammable.	(DIN EN 22719; ISO 2719)
Evaporation rate:	not applicable	
Flammability:	does not ignite	
Lower explosion limit:	not determined	
Upper explosion limit:	not determined	
Ignition temperature:	537 °C	(DIN EN 14522)
Vapour pressure:	approx. 23.3 hPa (20 °C) Information applies to the solvent.	
Density:	approx. 1.25 g/cm3 (20 °C)	
Relative vapour density (air):	not determined	
Solubility in water:	fully soluble	

Information on: bentazone
Partitioning coefficient n-octanol/water (log Kow): 0.77
(22 °C; pH value: 5)

Thermal decomposition: not determined
Viscosity, dynamic: 20.4 mPa.s
(20 °C)
Explosion hazard: not explosive
Fire promoting properties: not fire-propagating

Other information

Other Information:
If necessary, information on other physical and chemical parameters is indicated in this section.

10. Stability and Reactivity

Reactivity
No hazardous reactions if stored and handled as prescribed/indicated.

Chemical stability
The product is stable if stored and handled as prescribed/indicated.

Possibility of hazardous reactions
No hazardous reactions if stored and handled as prescribed/indicated.

Conditions to avoid
See MSDS section 7 - Handling and storage.

Incompatible materials

Substances to avoid:
strong acids, strong bases, strong oxidizing agents

Hazardous decomposition products

Hazardous decomposition products:
No hazardous decomposition products if stored and handled as prescribed/indicated.

11. Toxicological Information

Information on toxicological effects

Acute toxicity

Assessment of acute toxicity:
Of moderate toxicity after single ingestion. Virtually nontoxic after a single skin contact. Virtually nontoxic by inhalation.

Experimental/calculated data:
LD50 rat (oral): 1,260 mg/kg (OECD Guideline 401)
The product has not been tested. The statement has been derived from products of a similar structure or composition.

LC50 rat (by inhalation): > 7.1 mg/l 4 h (OECD Guideline 403)
The product has not been tested. The statement has been derived from products of a similar structure or composition.

LD50 rat (dermal): > 2,000 mg/kg (OECD Guideline 402)
The product has not been tested. The statement has been derived from products of a similar structure or composition.

Irritation

Assessment of irritating effects:
Not irritating to the eyes. Not irritating to the skin.

Experimental/calculated data:
Skin corrosion/irritation rabbit: non-irritant (OECD Guideline 404)
The product has not been tested. The statement has been derived from products of a similar structure or composition.

Serious eye damage/irritation rabbit: non-irritant (OECD Guideline 405)

Respiratory/Skin sensitization

Assessment of sensitization:
Sensitization after skin contact possible.

Experimental/calculated data:
Guinea pig maximization test guinea pig: skin sensitizing (OECD Guideline 406)
The product has not been tested. The statement has been derived from products of a similar structure or composition.

Germ cell mutagenicity

Assessment of mutagenicity:
The product has not been tested. The statement has been derived from the properties of the individual components. Mutagenicity tests revealed no genotoxic potential.

Carcinogenicity

Assessment of carcinogenicity:
The product has not been tested. The statement has been derived from the properties of the individual components. The results of various animal studies gave no indication of a carcinogenic effect.

Reproductive toxicity

Assessment of reproduction toxicity:
The product has not been tested. The statement has been derived from the properties of the individual components. The results of animal studies gave no indication of a fertility impairing effect.

Developmental toxicity

Assessment of teratogenicity:
The product has not been tested. The statement has been derived from the properties of the individual components. Animal studies gave no indication of a developmental toxic effect at doses that were not toxic to the parental animals.

Repeated dose toxicity and Specific target organ toxicity (repeated exposure)

Assessment of repeated dose toxicity:
The product has not been tested. The statement has been derived from the properties of the individual components. No substance-specific organotoxicity was observed after repeated administration of high doses to animals.

Other relevant toxicity information

Misuse can be harmful to health.

12. Ecological Information

Toxicity

Assessment of aquatic toxicity:
Acutely harmful for aquatic organisms. May cause long-term adverse effects in the aquatic environment.

The product has not been tested. The statement has been derived from the properties of the individual components.

Toxicity to fish:
LC50 (96 h) > 100 mg/l, Oncorhynchus mykiss (EPA 72-1)

Aquatic invertebrates:
EC50 (48 h) > 100 mg/l, Daphnia magna (OECD Guideline 202, part 1)

Aquatic plants:
EC50 (72 h) 33.3 mg/l, Pseudokirchneriella subcapitata (OECD Guideline 201)

EC50 (7 d) 25.3 mg/l, Lemna gibba (OECD guideline 221)

Persistence and degradability

Assessment biodegradation and elimination (H2O):
The product has not been tested. The statement has been derived from the properties of the individual components.

Information on: bentazone
Assessment biodegradation and elimination (H2O):
Not readily biodegradable (by OECD criteria). ----------

Bioaccumulative potential

Assessment bioaccumulation potential:
The product has not been tested. The statement has been derived from the properties of the individual components.

Information on: bentazone
Bioaccumulation potential:
Because of the n-octanol/water distribution coefficient (log Pow) accumulation in organisms is not to be expected.

Mobility in soil (and other compartments if available)

Assessment transport between environmental compartments:
The product has not been tested. The statement has been derived from the properties of the individual components.

Information on: bentazone
Assessment transport between environmental compartments:
Following exposure to soil, the product trickles away and can - dependant on degradation - be transported to deeper soil areas with larger water loads.

Results of PBT and vPvB assessment

The product does not contain a substance fullfilling the PBT (persistent/bioaccumulative/toxic) criteria or the vPvB (very persistent/very bioaccumulative) criteria.

Other adverse effects

The product does not contain substances that are listed in Annex I of Regulation (EC) 2037/2000 on substances that deplete the ozone layer.

Additional information

Other ecotoxicological advice:
Do not discharge product into the environment without control.

13. Disposal Considerations

Waste treatment methods

Must be disposed of or incinerated in accordance with local regulations.

Contaminated packaging:
Contaminated packaging should be emptied as far as possible and disposed of in the same manner as the substance/product.

14. Transport Information

Land transport

ADR

Not classified as a dangerous good under transport regulations

RID

Not classified as a dangerous good under transport regulations

Inland waterway transport
ADN

Not classified as a dangerous good under transport regulations

Sea transport
IMDG

Not classified as a dangerous good under transport regulations

Air transport
IATA/ICAO

Not classified as a dangerous good under transport regulations

15. Regulatory Information

Safety, health and environmental regulations/legislation specific for the substance or mixture

If other regulatory information applies that is not already provided elsewhere in this safety data sheet, then it is described in this subsection.

Chemical Safety Assessment

Advice on product handling can be found in sections 7 and 8 of this safety data sheet.

16. Other Information

Full text of the classifications, including the indication of danger, the hazard symbols, the R phrases, and the hazard statements, if mentioned in section 2 or 3:

Xn	Harmful.
22	Harmful if swallowed.
43	May cause sensitization by skin contact.
Acute Tox.	Acute toxicity
Eye Dam./Irrit.	Serious eye damage/eye irritation
Skin Sens.	Skin sensitization
Aquatic Chronic	Hazardous to the aquatic environment - chronic
H302	Harmful if swallowed.
H317	May cause an allergic skin reaction.

The data contained in this safety data sheet are based on our current knowledge and experience and describe the product only with regard to safety requirements. The data do not describe the product's properties (product specification). Neither should any agreed property nor the suitability of the product for any specific purpose be deduced from the data contained in the safety data sheet. It is the responsibility of the recipient of the product to ensure any proprietary rights and existing laws and legislation are observed.

Vertical lines in the left hand margin indicate an amendment from the previous version.

3.8 Procedures for destruction and decontamination

3.8.1 Pyrolytic behaviour under controlled conditions at 800°C

The halogen content of bentazone TK is less than 60 %. Approximately 800°C or higher temperatures are advised as incineration temperature. Expected combustion products are CO/CO_2, H_2O, SO_2 and N_2/NO_x.

3.8.2 Detailed instructions for safe disposal

Unwanted amounts of bentazone TK can be destroyed best by combustion in a licensed incinerator, in accordance with local regulations.

Contaminated packs and/or equipment that cannot be cleaned by washing with water should be disposed of in the same manner as the product.

3.8.3 Methods other than controlled incineration for disposal of packages

Combustion in a licensed incinerator is the only disposal recommended, if bentazone TK can not be used according to its purpose: the production of herbicides.

3.9 Procedures for decontamination of water in case of an accident

This study was submitted in the previous EU-authorization, and reviewed.

In the initial dossier, the report is listed under Annex point II A 3.6/1

Report:	II A 3.6/1 (new assignment would be II A 3.9/1) Schenk W. 1994 Possible procedures for the decontamination of water from Bentazone BASF DocID 1994/10410
Guidelines:	none
GLP:	No

Principle of determination:

The adsorbancy of organic compounds on activated carbon is evaluated by means of the adsorption isotherm according to Freundlich.

Summary of findings:

Bentazone is to be classified as moderately adsorbable at pH 7mainly because of its Freundlich-exponent of > 0.4. Under acidic conditions the adsorbancy considerably imporves.

Emergency measure in case of an accident:

Contaminated solid: Incineration.

Contaminated liquid: In the case of contamination of water, the undissolved amount of the product has to be separated by appropriate measures (e.g. filtration, centrifugation, extraction). The separated solid or the organic phase should be incinerated.
The aqueous phase has to be treated with approximately 5000 mg/l of activated powdered carbon at pH 3 for at least 12 hours. The separated activated carbon should be incinerated, too. The treated water (pH 6.5 – 9) is to be introduced into a public sewer leading to a public owned wastewater treatment works (POTW).

3.10 Other/special studies

This is not required by Regulation (EU) No 545/2011.

BASF
The Chemical Company

DOSSIER FOR THE EVALUATION OF THE ACTIVE SUBSTANCE

Bentazone (BAS 351 H)

Data requirement according to Regulation (EU) No. 544/2011 and (EU) No. 545/2011 (formerly Annex II and III of Directive 91/414/EEC) using OECD Guidance for Industry Data Submissions on Plant Protection Products and their Active Substance
(Dossier Guidance)

Document M-II

Summary and evaluation (Tier II)

Section 2

Analytical methods

BASF DocID 2014/1046512

compiled by

[redacted]

Date: 03 February 2014

4 Analytical Methods and Validation

List of Contents

In the following chapters, sometimes a reference to the Full Report (1998) is given. The full citation to this document reads "Full Report of the Peer Review Meetings organised by the ECCO teams for Bentazone, 1998".

According to Article 1(c) of Regulation (EU) No. 1141/2010 the supplementary dossier includes data and risk assessments which were not part of the original dossier and which are necessary to reflect changes. Reasoning for each test or study as required by Art 1(d)/(e) of the Regulation is given in the reference list of the dossier.

4.1 Analytical standards and samples

4.1.1 Analytical standards for pure active substance

Analytical standards for pure active substance are available from commercial suppliers.

4.1.2 Samples of the active substance as manufactured

Analytical standards of technical grade active substance are available upon request.

4.1.3 Analytical standards for relevant metabolites and other components

Analytical standards for relevant metabolites and other components are available upon request.

4.1.4 Samples of reference substances for relevant impurities

1,2-Dichloroethane is commonly available.

4.2 Methods for the analysis of the active substance as manufactured

4.2.1 Methods for the analysis of the active substance as manufactured

Report: II A 4.2.1/1
Stickland L.J. 2011(a)
Analytical method APL0631/01 - Determination of active ingredient Bentazone in Bentazone TC, Bentazone TK and formulations containing Bentazone
BASF DocID 2011/1074529
Guidelines: None
GLP: No

Analytical method APL631/01 is used to determine the bentazone content in technical material, technical concentrate and BAS 351 32 H soluble concentrate (SL) formulations. The samples are extracted into methanol/ammonium acetate buffer solution then separated by reversed phase HPLC using a C18 column. Detection is done by UV absorbance at 340 nm and quantitation by external standard.

The fortification experiments demonstrate, that analytical method APL0631/01 is applicable to determine the contents of bentazone in bentazone TC (precipitated acid), in bentazone TK and in formulations of the type SL.

4.2.2 Applicability of existing CIPAC methods

A CIPAC method for the determination of bentazone in technical concentrate and in formulations is available (Bentazone, 366/TC/M, 1984). However, as the method is rather old the equipment (columns) is not available anymore. Analytical method APL0631/01 has therefore been developed in order to meet the current state of the art.

4.2.3 Description of analytical methods for the determination of impurities

This confidential information is provided in Document J.

4.2.4 Description of analytical methods for the determination of additives

This confidential information is provided in Document J.

4.2.5 Enforcement analytical methodology

Report:	II A 4.2.5/1 Stickland L.J. 2011(b) Validation of analytical method APL0631/01: Determination of active ingredient Bentazone in Bentazone TC, Bentazone TK and formulations containing Bentazone BASF DocID 2011/1074530
Guidelines:	EEC 91/414 Annex II; SANCO/3030/99 rev. 4 (11 July 2000); EPA 830.1800
GLP:	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Validation
Full validation of analytical method APL0631/01 has been conducted.

The validation data of method APL0631/01 with respect to accuracy, precision, linearity and specificity prove that the method is suitable to determine the content of bentazone in the technical concentrate (BAS 351 56 H) as well as in the precipitated dry active ingredient .

Enforcement analytical methodology:

Accuracy:

Known amounts of the reference item bentazone were weighed six times in each case into the blank technical concentrate of BAS 351 AW H (blank formulation of TK) and analyzed according to the analytical method APL0631/01. The six spiked solutions were prepared in 50 %-, 100 % and 125 % levels according to the target concentration of the active in the test item. There were no stragglers or outliers detected.

The analysis yielded for the 50 % level 100 %, for the 100 % level 101 % and for the 125 % level 102 % recovery of the expected values.
These values demonstrates that the observed results correspond in a high degree to the true values of the analyte in the sample.

Precision:

A sample of BAS 351 H TC (precipitated acid, batch N 187) was weighed six times. The contents of the active substance bentazone were determined according to the analytical method presented here. There were no stragglers or outliers detected.
The analysis yielded a mean value of 98.6 % (w/w) bentazone with a relative standard deviation of 0.40 %.

A production sample of bentazone technical concentrate (BAS 351 56 H, batch no. B135-1/9) was weighed six times. The contents of the active substance bentazone were determined according to the analytical method presented here. There were no stragglers or outliers detected.
The analysis yielded a mean value of 51.5 % (w/w) bentazone with a relative standard deviation of 0.70 %.

The values are situated in a range, which can be expected by the used equipment and the analytical method in general. The RSD values determined meet the requirements (RSDr < RSDR x 0.67) given by the modified Horwitz equation.

Linearity:

The linearity of detector response was determined by preparing five solutions of bentazone over the approximate concentration range of 75 – 125 % of the working standard concentration. These samples were injected in duplicate.
There were no stragglers or outliers detected.
The results are shown in the following table:

Active substance	correlation coefficient	slope	intercept
Bentazone	0.9999	1932.7	10.861

This test demonstrates linearity of response to the analyte concentration over the practical range of the method.

Specificity:

The specificity of the method was demonstrated by spectrometric examination, i.e. by UV-spectra and MS. It was verified by comparing the particular retention times of the reference items with that of the test items. The blank formulation for the technical concentrate was also checked. The HPLC chromatograms do not show any further substances at the place of the peaks of the active ingredients.

Conclusion:

The fortification experiments demonstrate, that analytical method APL0631/01 is applicable to determine the contents of bentazone in bentazone TC (precipitated acid), in bentazone TK and in formulations of the type SL.

4.2.6 Inter-Laboratory analytical methodology validation

Method APL0631/01 is not inter-laboratory validated.

4.2.7 Storage stability of working solutions in analytical methodology

Storage stability of working solutions is not required for the methods for the determination of active substance and impurities in technical active ingredient.

4.3 Description of analytical methods for the determination of residues

The following residue definition for both risk assessment and monitoring has been established for **products of plant origin** (according to Regulation (EC) No 396/2005 as amended by Regulation (EU) No 893/2010):

Bentazone (sum of bentazone and the conjugates of 6-OH-bentazone and 8-OH-bentazone expressed as bentazone).

For products of animal origin, the residue definition for both risk assessment and monitoring according to Regulation (EC) No 396/2005 as amended by Regulation (EU) No 893/2010 is bentazone only. In context of this dossier it is proposed to change the residue definition for both risk assessment and monitoring for **products of animal origin** to:

Bentazone (sum of bentazone and the conjugates of 6-OH-bentazone and 8-OH-bentazone expressed as bentazone).

In context of the initial Annex I inclusion various analytical methods were submitted to Germany as Rapporteur Member State and were considered adequate. Additional data on validation of state-of-the-art methods for analysis of bentazone and the conjugates of 6-OH- and 8-OH-bentazone in plant and animal matrices are provided in this dossier.

No endpoints regarding analytical methods were specified by EC in the Review Report (7585/VI/97 - final, 30. November 2000), and no further studies were identified which were necessary for Annex I inclusion.

The following list of endpoints was copied from the Full Report (1998).

Analytical methods for residues (Annex IIA, point 4.2)

Food/feed of plant origin (principle of method and LOQ for methods for monitoring purposes)	GC-FPD; LOQ: 0.02-0.05 mg/kg (note: additional validation data may be required for 0.02 mg/kg) GC-PND; LOQ: 0.05 mg/kg Bentazone, 6-OH- and 8-OH-bentazone (incl. their conjugates) can be determined
Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes)	GC-FPD; LOQ: 0.02 mg/kg milk, 0.05 mg/kg (other) Bentazone: milk, eggs, muscle, liver, kidney, fat 6-OH- and 8-OH-bentazone: eggs, muscle, liver.
Body fluids and tissues (principle of method and LOQ)	Not required, classification Xn

LOQ = limit of quantitation

Table 4.3/1 EU Conclusions: Analytical methods for residues of bentazone in food of plant or animal origin

Crop/Matrix	EU Agreed Method (Full Report* - 1998)	Proposed Method**
Food of plant origin (risk assessment)	438/0 (GC-MS) 438/2 (LC-MS/MS)#	438/2 (LC-MS/MS)
Food of plant origin (MRL enforcement)	GC-FPD 438/2 (LC-MS/MS)#	438/2 (LC-MS/MS)
Food of animal origin (risk assessment)	Not specified	438/2 (LC-MS/MS)
Food of animal origin (MRL enforcement)	GC-FPD	438/2 (LC-MS/MS)

* Full Report of the Peer Review Meetings organised by the ECCO teams for Bentazone
** Since Annex I inclusion new studies on the a.s. have been performed and as a result new methods are proposed in the present dossier.
# EFSA Reasoned Opinion, EFSA Journal 2011;9(5):2188

Data on the new enforcement Method No. 438/2 has been peer reviewed in context of MRL modifications and was considered appropriate (EFSA Journal 2011;9(5):2188). Validation of Method No. 438/1 and the applicability of the enzymatic cleavage step in Methods No. 438/1 and 438/2 have not been peer reviewed yet. Summaries of all analytical methods developed and validated since last Annex I inclusion are presented below.

Plant Matrices

A high extractability of bentazone and the conjugates of 6-OH-bentazone and 8-OH-bentazone with organic solvents such as methanol from plant matrices was confirmed in a wheat metabolism study submitted in the context of the present dossier (see M-II, 6.2).

Table 4.3/2 Summary table of analytical methods used for the quantitation of bentazone and its metabolites 8-OH-bentazone and 6-OH-bentazone in plant matrices

Group	Crop / Matrix	Analyte(s)	Method BASF Method No.	LOQ	Reference
Cereal Crops Oil seeds Vegetables	wheat, barley, maize soya, flax potato bean, pea, onion	bentazone 8-OH-bentazone 6-OH-bentazone	GC-MS 438/1 (validation)	0.02 mg/kg for bentazone and its two metabolites	2000/1000243 II A 4.3/1
N/A (enzymatic cleavage in 438/1)	N/A	bentazone 8-OH-bentazone 6-OH-bentazone	HPLC-UV	N/A	2005/1027613 II A 4.3/2
Cereal Crops Fruit	maize orange soybean onion	bentazone 8-OH-bentazone 6-OH-bentazone	LC-MS/MS 438/2 (validation)	0.01 mg/kg for bentazone and its two metabolites	2007/1013924 II A 4.3/3
Cereal Crops Fruit Vegetables	maize orange soybean, onion	bentazone 8-OH-bentazone 6-OH-bentazone	LC-MS/MS 438/2 (ILV)	0.01 mg/kg for bentazone and its two metabolites	2007/1013926 II A 4.3/4

N/A Not applicable

Report: II A 4.3/1
Linder G. et al. 2000(c)
Technical procedure - Method for the determination of Bentazon, 6-OH-Bentazon and 8-OH-Bentazon in plant matrices - Method 438/1
BASF DocID 2000/1000243

Guidelines: none

GLP: No

Principle of the method: BASF Method No. 438/1 allows the determination of bentazone and all sugar conjugates which can be hydrolysed to 6-OH-bentazone and 8-OH-bentazone. It is a miniaturised version of the previous procedure BASF Method No. 438/0 and was adapted to a number of additional crops. Bentazone and its metabolites are extracted from plant matrices with aqueous methanol. After purification of a 20% aliquot by an iso-octane liquid-liquid partition, the two relevant metabolites which are present as glucosides are hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic plant constituents, a reversed phase C_{18}-column clean-up is performed. The analytes are then methylated with diazomethane and their derivatives are purified using a silica gel-column. The final determination of the residues of bentazone and its OH metabolites is performed by GC-MS. The limit of quantitation (LOQ) of the method for bentazone, 6-OH-bentazone and 8-OH-bentazone is 0.02 mg/kg each.

Recovery findings: BASF Method No. 438/1 was developed for the determination of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in wheat (grain, straw, shoot, root, ear), potato (shoot, tuber), maize (shoot, root, ear, grain, straw), barley (shoot, ear, straw, grain), flax (shoot, straw, seed), bean (shoot, siliquaes, straw, seed), soya (shoot, straw, seed), onion (shallot, bulb) and pea (plant, fruit). The mean overall recoveries were at 91.9% for bentazone, 84.9% for 6-OH-bentazone and 81.4% for 8-OH-bentazone. The detailed results are given in Table 4.3/3.

Table 4.3/3 Recovery results of bentazone, 8-OH-bentazone and 6-OH-bentazone in plant matrices spiked with 0.02, 0.2 and 2.0 mg/kg

Crop	Matrix	Bentazone			6-OH-bentazone			8-OH-bentazone		
		0.02	0.2	2.0	0.02	0.2	2.0	0.02	0.2	2.0
		mg/kg			mg/kg			mg/kg		
wheat	shoots	114.9	-	82.4	81.3	-	59.7	92.1	-	59.9
	shoots	112.7	-	105.4	89.2	-	80.2	112.1	-	84.8
	ears	104.1	-	100.5	87.0	-	82.6	71.7	-	-
	straw	109.3	103.4	-	98.1	90.2		78.5	79.4	-
	grain	121.4	97.1	88.1	109.3	101.3	76.8	96.3	-	-
	roots	98.1	111.7	107.4	75.7	89.7	101.8	91.5	87.6	75.9
potato	shoots	-	-	-	81.1		-	82.2	-	-
	tuber	81.4	91.2	-	57.2	71.7	-	86.1	71.4	-
potato	tuber	75.6	76.2	-	90.0	74.7	-	88.4	69.0	-
	tuber	78.7	-	-	77.9	-	-	110.4	-	-
maize	shoots	-	94.9	-	-	64.8	-	-	55.4	-
	shoots	90.0	64.1	-	70.1	54.6	-	103.4	57.5	-
	roots	120.1	78.9	90.9	87.8	70.9	88.8	98.1	71.8	84.6
	roots	91.6	89.5	75.4	88.9	67.2	68.7	116.9	73.8	57.4
	roots	110.4	67.4	74.8	105.5	72.3	75.1	108.6	61.1	62.2
	ears [1)]	70.0	72.2	-	76.4	63.4	-	107.3	64.3	-
	straw	76.8	82.3	-	100.7	86.3	-	81.5	69.8	-
	grain	100.7	89.7	-	92.2	88.7	-	84.6	75.1	-
barley	shoots	90.7	-	103.5	67.0	-	102.5	69.5	-	77.8
	shoots	89.0	-	82.2	86.0	-	95.7	89.7	-	77.6
	ears	90.0	-	103.9	103.1	-	101.6	115.8	-	85.0
	straw	92.8	-	98.7	70.5	-	111.7	114.4	-	87.1
	grain	95.0	-	69.4	108.1	69.6		117.7	-	60.8
flax	shoots	97.3	65.5	81.0	-	58.0	74.5	113.1	-	60.2
	straw	94.4	103.7	-	81.6	88.6	-	82.1	78.5	-
	seed	116.7	100.1	-	103.8	86.5	-	98.2	84.5	-
bean	seed	81.1	96.0	-	86.5	89.5	-	108.1	73.8	-
	straw	77.1	88.1	-	95.6	92.0	-	68.1	69.8	-
	shoots	51.5	74.3	87.5	63.7	81.5	80.6	55.3	74.5	68.6
	siliquaes	-	80.4	-	94.6	-		-	71.9	
soya	shoots	-	73.9	93.1	-	87.2	100.0	-	-	84.0
	seed	110.7	85.2	-	89.9	69.7	-	-	-	-
	straw	106.9	76.7	-	83.7	71.4	-	-	-	-
onion	shallot	75.8	99.0	-	79.6	83.1	-	70.4	70.7	-
	bulb	80.1	92.9	-	60.6	80.3	-	61.9	-	52.9
onion	shallot	103.4	105.6	-	66.4	75.5	-	70.4	-	-
	bulb	94.1	99.5	-	89.8	107.0	-	100.0	95.5	-
bean	shoots	98.2	-	90.7	98.2	-	90.9	121.3	-	78.1
	beans [2)]	107.8	97.4	-	95.2	74.3	-	117.0	72.3	-
	dry bean	112.7	89.5	-	99.9	70.1	-	119.9	75.8	-
	straw [2)]	116.6	90.7	-	124.3	80.8	-	123.9	79.7	-
pea	plant [3)]	-	-	101.8	120.4	-	91.0	-	116.4	72.4
	fruit	114.5	115.4	-	83.7	107.6	-	94.5	117.9	-
max (%):		121.4	115.4	107.4	124.3	107.6	111.7	123.9	117.9	87.1
min (%):		51.5	64.1	69.4	57.2	54.6	59.7	55.3	55.4	52.9
mean (%):		**96.1**	**88.8**	**90.9**	**88.0**	**79.6**	**87.2**	**95.2**	**76.7**	**72.3**
SD (+/-):		16.2	13.4	11.7	15.3	13.1	14.0	18.8	14.9	11.4
RSD (+/-):		16.9	15.1	12.9	17.4	16.5	16.1	19.7	19.4	15.8

1) with husks, 2) with pods, 3) without roots

Linearity: Good linearity was observed in the range of 0.0025 to 0.5 µg/mL for *N*-methylbentazone, 8-methoxy-*N*-methylbentazone and 6-methoxy-*N*-methylbentazone (external reference standard).

Specificity: The method determines bentazone, 8-OH-bentazone and 6-OH-bentazone in plant matrices. There were no known interferences from plant components or from reagents, solvents and glassware used.

Limit of Quantitation: The limit of quantitation was defined by the lowest fortification level successfully tested and was 0.02 mg/kg in the maize matrices forage, grain and straw.

Repeatability: The relative standard deviation (RSD) was below 20% for bentazone, 6-OH-bentazone and 8-OH-bentazone. The detailed values are shown in Table 4.3/3.

Reproducibility: The reproducibility of the method was not estimated as identical samples were not evaluated by an independent laboratory. However, based on the performance of the method, its reproducibility is expected to be good.

Conclusion: BASF Method No. 438/1 was developed for determination of bentazone and its 6-OH and 8-OH metabolites in the maize matrices forage, grain and straw down to the limit of quantitation of 0.02 mg/kg. So far no interference with sample matrices of other compounds was detected.

Report: II A 4.3/2
Mackenroth C., Kerl W. 2005(d)
Applicability of an enzymatic cleavage in analytical method No. 438/1 used to determine bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone as glucoside derivatives
BASF DocID 2005/1027613

Guidelines: None

GLP: No, not subject to GLP regulations

Principle of the method: In this statement data are presented which demonstrate the applicability of the enzymatic cleavage and its comparability to the acidic treatment. The data were generated in a study performed earlier under GLP in order to investigate the storage stability of bentazone, and the glucoside derivatives of 6-OH-bentazone and 8-OH-bentazone. The glucoside derivatives were produced in wheat and rape cell suspension cultures since previous attempts to produce the glucosides chemically were not successful. In case of the 8-OH derivative, also the cell culture production showed poor yield. Therefore, an attendant test is presented with the conjugated 6-OH isomer. As it was the purpose of the study to prove the stability of the glucosides, it was also necessary to gain data about their hydrolysis products in order to be able to recognise and follow the reaction. Therefore, data were produced in order to characterise and identify the 6-OH glucoside and the corresponding free OH compound (as model compound for the two isomers). For that purpose, the 6-OH glucoside was subjected to an enzymatic cleavage under the same conditions as described in Method No. 438/1 and 438/2. To the 6-OH bentazone glucoside obtained from cell suspension cultures a mixture of ß-glucosidase and hesperidinase (1+1) and a solution of ascorbic acid were added as described in the technical procedure of method 438/1. After adjusting to a defined weight with water, the reaction mixture was kept at 37°C for 14 hours. The reaction was performed without as well as in presence of maize seed matrix. The reaction mixture was analysed by means of HPLC equipped with a radioactivity monitor and UV detector.

Recovery findings: The results show that the two compounds 6-OH-bentazone and 6-OH-bentazone glucoside are clearly distinguishable under these conditions. The enzymatic cleavage reaction shows that no traces of the glucoside are detectable. Therefore, the reaction can be regarded to proceed uniformly and quantitatively and is not influenced by the presence of matrix constituents.

Linearity: Not applicable

Specificity: Not applicable

Limit of Quantitation: Not applicable

Repeatability: Not applicable

Reproducibility: Not applicable

Conclusion: As the enzymatic cleavage reaction proved to be quantitative, it can be concluded that the conversion is equivalent to the hydrolysis by acid used in the past. There are no reasons to presume that the corresponding reaction of the 8-OH-glucoside (which could not be followed due to insufficient yield) should proceed differently. Therefore, Method No. 438/1 and 438/2 can be regarded as valid. Also the applicability of the method in matrices such as soybean can be verified by the experiments shown: the presence of maize seed matrix (which is comparable to soybean regarding its oil content) did not influence the conversion of 6-OH-bentazone glucoside.

Report: II A 4.3/3
Class T. 2007(c)
Bentazone and its two OH-metabolites: Validation of residue method 438/2 for plant materials using LC/MS/MS
BASF DocID 2007/1013924

Guidelines: EEC 91/414 Annex II (Part A Section 4.2); SANCO/825/00 rev. 7 (17 March 2004); SANCO/3029/99 rev. 4 (11 July 2000)

GLP: Yes
(laboratory certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Principle of the method: Extraction and enzymatic cleavage of residues of bentazone and its conjugated 6-OH- and 8-OH-metabolites from plant specimen was adopted from BASF Method No. 438/2 which uses GC-MS for the determination of the analytes in plant materials: bentazone and its hydroxy-metabolites present in plant as glucosides were extracted from plant material with aqueous methanol. After purification of an aliquot by liquid-liquid partition with iso-octane, glucosides were cleaved enzymatically to 6-OH-bentazone and 8-OH-bentazone. After $Ca(OH)_2$-precipitation of acidic plant constituents, a reversed phase C_{18}-SPE clean-up was performed. Final determination of bentazone and its OH-metabolites was performed by LC-MS/MS, monitoring two parent-daughter ion transitions for each compound.

Recovery findings: Untreated samples of maize silage, maize grain, whole orange, soybean and onion were fortified with 0.01 and 0.1 mg/kg bentazone, 6-OH-bentazone and 8-OH-bentazone. In all matrices, the mean recoveries were between 72% and 107%. Detailed results are given in Table 4.3/4.

Table 4.3/4 Recovery results of bentazone, 8-OH-bentazone and 6-OH-bentazone in plant matrices

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)		Relative Standard Deviation (%)	
Transition				**239 m/z > 132 m/z**	**239 m/z > 175 m/z**	**239 m/z > 132 m/z**	**239 m/z > 175 m/z**
bentazone	Maize Silage	0.01	5	100	99	2	1
		0.1	5	96	95	5	6
	Maize Grain	0.01	5	92	96	5	5
		0.1	5	89	88	2	3
	Whole Orange	0.01	5	99	101	2	3
		0.1	5	107	106	9	10
	Soybean	0.01	5	89	88	3	3
		0.1	5	90	90	4	5
	Onion	0.01	5	89	89	7	6
		0.1	5	94	94	4	5
Transition				**255 m/z > 213 m/z**	**255 m/z > 148 m/z**	**255 m/z > 213 m/z**	**255 m/z > 148 m/z**
6-OH-bentazone	Maize Silage	0.01	5	88	90	4	2
		0.1	5	86	85	4	3
	Maize Grain	0.01	5	83	87	5	3
		0.1	5	92	91	4	2
	Whole Orange	0.01	5	98	98	2	1
		0.1	5	94	96	6	6
	Soybean	0.01	5	82	100	11	6
		0.1	5	98	99	3	3
	Onion	0.01	5	85	87	9	8
		0.1	5	94	92	7	8
Transition				**255 m/z > 148 m/z**	**255 m/z > 191 m/z**	**255 m/z > 148 m/z**	**255 m/z > 191 m/z**
8-OH-bentazone	Maize Silage	0.01	5	80	79	1	2
		0.1	5	75	76	5	5
	Maize Grain	0.01	5	76	73	6	6
		0.1	5	82	81	6	6
	Whole Orange	0.01	5	100	102	3	1
		0.1	5	84	83	4	4
	Soybean	0.01	5	72	73	6	6
		0.1	5	88	87	3	2
	Onion	0.01	5	74	73	5	6
		0.1	5	87	87	10	11

Linearity: Good linearity was observed in the range between 0.10 and 10.0 ng/mL.

Specificity: No interferences with sample matrices were reported.

Limit of Quantitation: The limit of quantitation was defined by the lowest fortification level successfully tested which was 0.01 mg/kg for all examined matrices.

Repeatability: The coefficients of variation with respect to recoveries following fortifications at the limit of quantitation were between 1% and 11% in all examined matrices. The values obtained are indicative of the method having satisfactory repeatability.

Reproducibility: The reproducibility of the method was evaluated by an independent laboratory (see report II A 4.3/4, BASF DocID 2007/1013926). Based on the performance of the method, also in the second laboratory, the reproducibility is very good.

Conclusion: The study validated the accurate determination of bentazone, 6-OH-bentazone and 8-OH-bentazone by using BASF Method No. 438/2 in maize silage, maize grain, whole orange, soy bean and onion plant specimen. The limit of quantitation was 0.01 mg/kg for all commodities tested.

Report: II A 4.3/4
Schulz H., Meyer M. 2007(e)
Determination of Bentazone, 6-OH-Bentazone and 8-OH-Bentazone in plant matrices - Independent laboratory validation of the analytical method No. 438/2
BASF DocID 2007/1013926

Guidelines: OECD-DOC ENV/MC/CHEM(98)17 Paris 1998; SANCO/825/00 rev. 7 (17 March 2004); SANCO/3029/99 rev. 4 (11 July 2000); Residue analytical methods for post-registration control purposes (July 21 1998) Federal Biological Research Centre for Agriculture and Forestry Braunschweig Germany; Guidance Document of Residue Analytical Methods 8064/VI/97 rev. 4 15.12.1998

GLP: Yes
(laboratory certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Principle of the method: Bentazone and its metabolites are extracted from plant or animal matrices with aqueous methanol. After purification of an aliquot by an iso-octane liquid-liquid partition, the two relevant metabolites which are present as glucosides were hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic matrix constituents, a reversed phase C_{18}-SPE clean-up is performed. The final determination of bentazone and its hydroxy-metabolites is performed by LC-MS/MS, monitoring two parent-daughter ion transitions for each compound.

Recovery findings: Untreated samples of maize silage, maize grain, lemon, soybean and onion) were fortified with 0.01 and 0.1 mg/kg bentazone, 6-OH-bentazone and 8-OH-bentazone. In all matrices, the mean recoveries were between 71.6% and 108.2%. Results are summarised in Table 4.3/5.

Table 4.3/5 Recovery results of bentazone, 8-OH-bentazone and 6-OH-bentazone in plant matrices

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)		Relative Standard Deviation (%)	
Transition				**239 m/z > 132 m/z**	**239 m/z > 175 m/z**	**239 m/z > 132 m/z**	**239 m/z > 175 m/z**
bentazone	Lemon	0.01	5	89.9	90.4	1.1	1.7
		0.1	5	79.4	79.0	3.3	3.8
	Onion	0.01	5	98.4	98.8	0.6	0.9
		0.1	5	100.6	99.6	1.1	1.7
	Soybean	0.01	5	100.3	99.1	1.8	1.9
		0.1	5	101.4	101.0	1.3	1.6
	Maize Silage	0.01	5	102.5	102.2	3.1	2.2
		0.1	5	98.9	99.2	1.0	0.7
	Maize Grain	0.01	5	97.5	98.0	3.5	2.9
		0.1	5	97.9	98.5	2.1	1.6
Transition				**255 m/z > 213 m/z**	**255 m/z > 148 m/z**	**255 m/z > 213 m/z**	**255 m/z > 148 m/z**
6-OH-bentazone	Lemon	0.01	5	90.1	90.9	2.9	2.5
		0.1	5	108.2	107.4	10.6	9.5
	Onion	0.01	5	83.0	82.4	1.7	1.8
		0.1	5	71.6	77.6	3.9	4.0
	Soybean	0.01	5	102.0	90.6	2.9	1.6
		0.1	5	83.2	79.4	2.2	2.1
	Maize Silage	0.01	5	94.4	93.4	4.5	4.4
		0.1	5	76.6	77.9	1.9	2.2
	Maize Grain	0.01	5	87.4	89.0	4.4	6.1
		0.1	5	74.8	75.6	1.5	0.8
Transition				**255 m/z > 148 m/z**	**255 m/z > 191 m/z**	**255 m/z > 148 m/z**	**255 m/z > 191 m/z**
8-OH-bentazone	Lemon	0.01	5	84.6	84.3	1.6	2.7
		0.1	5	95.9	95.7	5.1	5.4
	Onion	0.01	5	100.6	101.2	1.6	1.3
		0.1	5	84.6	85.1	1.9	2.5
	Soybean	0.01	5	85.2	85.6	2.4	2.4
		0.1	5	99.6	98.4	0.6	1.2
	Maize Silage	0.01	5	97.2	96.6	12.4	12.6
		0.1	5	77.7	77.5	2.9	4.1
	Maize Grain	0.01	5	83.4	82.8	3.7	4.3
		0.1	5	88.7	88.5	1.9	1.2

Linearity: Good linearity was observed in the range between 0.10 and 5.0 ng/mL.

Specificity: No interferences with sample matrices were reported.

Limit of Quantitation: The limit of quantitation was defined by the lowest fortification level successfully tested which was 0.01 mg/kg for all examined matrices.

Repeatability: The coefficients of variation with respect to recoveries following fortifications at the limit of quantitation were between 0.6% and 12.6% in all examined matrices. The values obtained are indicative of the method having satisfactory repeatability.

Reproducibility: This independent laboratory validation proves a very good reproducibility of BASF Method No. 438/2.

Conclusion: The independent laboratory validation approved the accurate, repeatable and reproducible determination of bentazone, 6-OH-bentazone and 8-OH-bentazone using BASF Method No. 438/2 in maize silage and grain, lemon, soy bean and onion plant specimen. The limit of quantitation was 0.01 mg/kg for all commodities tested.
Therefore it is shown that BASF Method No. 438/2 fulfils all data requirements for enforcement and data generation.

Animal matrices

Table 4.3/6 Summary table of analytical methods used for the quantitation of bentazone and its metabolites 8-OH-bentazone and 6-OH-bentazone in animal matrices

Animal	Matrix	Analyte(s)	Method BASF Method No.	LOQ	Reference
Chicken Bovine	egg meat, liver, milk	bentazone 8-OH-bentazone 6-OH-bentazone	LC-MS/MS 438/2 (validation)	0.01 mg/kg for bentazone and its two metabolites	2007/1013925 II A 4.3/5
Chicken Bovine	egg meat, liver, milk	bentazone 8-OH-bentazone 6-OH-bentazone	LC-MS/MS 438/2 (ILV)	0.01 mg/kg for bentazone and its two metabolites	2007/1013927 II A 4.3/6

Report:	II A 4.3/5 Bacher R. 2007(c) Bentazone and its two OH-metabolites: Validation of residue method 438/2 for animal materials using LC/MS/MS BASF DocID 2007/1013925
Guidelines:	EEC 91/414 Annex II (Part A Section 4.2); SANCO/825/00 rev. 7 (17 March 2004); SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	Yes (laboratory certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Principle of the method: Extraction and enzymatic cleavage of residues of bentazone and its conjugated 6-OH- and 8-OH-metabolites from plant specimen was adopted from BASF Method 438/2 which uses GC/MS for the determination of the analytes in plant materials: bentazone and its OH-metabolites present in animal matrices as glucuronides or sulfates were extracted with aqueous methanol. After purification of an aliquot by liquid-liquid partition with iso-octane, glucuronides and sulfates were cleaved enzymatically to 6-OH-bentazone and 8-OH-bentazone. After $Ca(OH)_2$-precipitation of acidic matrix constituents, a reversed phase C_{18}-SPE clean-up was performed. The final determination of bentazone and its hydroxy-metabolites was performed by LC-MS/MS, monitoring two parent-daughter ion transitions for each compound.

Recovery findings: Animal specimen of whole milk and egg, bovine meat and liver were fortified with 0.01 and 0.1 mg/kg bentazone, 6-OH-bentazone and 8-OH-bentazone. In all matrices, the mean recoveries were between 73% and 106%. Detailed results are given in Table 4.3/7.

Table 4.3/7 Recovery results of bentazone, 8-OH-bentazone and 6-OH-bentazone in animal matrices

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)		Relative Standard Deviation (%)	
Transition				**239 m/z > 132 m/z**	**239 m/z > 175 m/z**	**239 m/z > 132 m/z**	**239 m/z > 175 m/z**
bentazone	Milk	0.01	5	102	100	7	7
		0.1	5	97	97	3	4
	Egg	0.01	5	102	105	3	2
		0.1	5	96	93	2	4
	Bovine meat	0.01	5	98	96	6	4
		0.1	5	98	95	5	6
	Bovine liver	0.01	5	106	103	5	4
		0.1	5	104	103	7	6
Transition				**255 m/z > 213 m/z**	**255 m/z > 148 m/z**	**255 m/z > 213 m/z**	**255 m/z > 148 m/z**
6-OH-bentazone	Milk	0.01	5	98	90	5	6
		0.1	5	89	88	12	11
	Egg	0.01	5	84	83	7	4
		0.1	5	80	80	6	5
	Bovine meat	0.01	5	81	77	6	3
		0.1	5	76	75	18	17
	Bovine liver	0.01	5	102	103	8	11
		0.1	5	103	106	4	3
Transition				**255 m/z > 148 m/z**	**255 m/z > 191 m/z**	**255 m/z > 148 m/z**	**255 m/z > 191 m/z**
8-OH-bentazone	Milk	0.01	5	78	80	4	4
		0.1	5	76	78	7	7
	Egg	0.01	5	79	77	3	4
		0.1	5	75	73	7	7
	Bovine meat	0.01	5	82	84	8	5
		0.1	5	78	79	16	14
	Bovine liver	0.01	5	90	89	7	9
		0.1	5	91	92	7	11

Linearity: Good linearity was observed in the range between 0.10 and 10.0 ng/mL.

Specificity: No interferences with sample matrices were reported.

Limit of Quantitation: The limit of quantitation was defined by the lowest fortification level successfully tested which was 0.01 mg/kg for all examined matrices.

Repeatability: The coefficients of variation with respect to recoveries following fortifications at the limit of quantitation were between 2 and 18% in all examined matrices. The values obtained are indicative of the method having satisfactory repeatability.

Reproducibility: The reproducibility of the method was proven by an independent laboratory validation (see DocID 2007/1013927 below).

Conclusion: The study validated the accurate determination of bentazone, 6-OH-bentazone and 8-OH-bentazone by using BASF Method No. 438/2 in whole milk, egg, bovine meat and liver specimen. The limit of quantitation was 0.01 mg/kg for all animal commodities tested.

Report: II A 4.3/6
Schulz H., Meyer M. 2007(f)
Determination of Bentazone, 6-OH-Bentazone and 8-OH-Bentazone in animal matrices - Independent laboratory validation of the analytical method. No. 438/2
BASF DocID 2007/1013927

Guidelines: OECD-DOC ENV/MC/CHEM(98)17 Paris 1998; SANCO/825/00 rev. 7 (17 March 2004); SANCO/3029/99 rev. 4 (11 July 2000); Residue analytical methods for post-registration control purposes (July 21 1998) Federal Biological Research Centre for Agriculture and Forestry Braunschweig Germany; Guidance Document of Residue Analytical Methods 8064/VI/97 rev. 4 15.12.1998

GLP: Yes
(laboratory certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Principle of the method: Bentazone and its metabolites are extracted from animal matrices with aqueous methanol. After purification of an aliquot by an iso-octane liquid-liquid partition, the two relevant metabolites which are present as glucosides were hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic matrix constituents, a reversed phase C_{18}-SPE clean-up is performed. The final determination of bentazone and its OH-metabolites is performed by LC-MS/MS, monitoring two parent-daughter ion transitions for each compound.

Recovery findings: Animal specimen of whole milk and egg, bovine meat and liver were fortified with 0.01 and 0.1 mg/kg bentazone, 6-OH-bentazone and 8-OH-bentazone, respectively. In all matrices, the mean recoveries were between 71.8% and 105.3%. Detailed results are given in Table 4.3/8.

Table 4.3/8 Recovery results of bentazone, 8-OH-bentazone and 6-OH-bentazone in animal matrices

Test Substance	Crop	Fortification Level (mg/kg)	No. of Tests	Average Recovery (%)		Relative Standard Deviation (%)	
Transition				**239 m/z > 132 m/z**	**239 m/z > 175 m/z**	**239 m/z > 132 m/z**	**239 m/z > 175 m/z**
bentazone	Bovine meat	0.01	5	88.3	88.6	0.5	0.7
		0.1	5	93.1	93.0	0.6	1.6
	Bovine liver	0.01	5	101.0	101.2	1.2	1.3
		0.1	5	97.7	98.6	1.2	1.8
	Milk	0.01	5	101.4	101.1	4.2	4.1
		0.1	5	97.0	97.3	4.9	3.9
	Egg	0.01	5	101.5	101.9	4.2	3.2
		0.1	5	103.7	103.4	1.9	1.7
Transition				**255 m/z > 213 m/z**	**255 m/z > 148 m/z**	**255 m/z > 213 m/z**	**255 m/z > 148 m/z**
6-OH-bentazone	Bovine meat	0.01	5	75.5	75.9	1.7	1.6
		0.1	5	73.5	75.4	1.9	0.6
	Bovine liver	0.01	5	87.8	86.2	5.0	4.3
		0.1	5	74.4	71.8	2.6	2.6
	Milk	0.01	5	89.8*	90.0*	1.9	2.7*
		0.1	5	85.3	85.7*	6.8	5.6*
	Egg	0.01	5	88.2	87.2	7.4	7.7
		0.1	5	92.7	91.0	6.1	6.1
Transition				**255 m/z > 148 m/z**	**255 m/z > 191 m/z**	**255 m/z > 148 m/z**	**255 m/z > 191 m/z**
8-OH-bentazone	Bovine meat	0.01	5	81.3	79.8	1.0	1.6
		0.1	5	81.1	80.5	1.7	0.8
	Bovine liver	0.01	5	105.3	104.4	3.0	3.1
		0.1	5	89.8	88.9	1.4	2.4
	Milk	0.01	5	88.6	87.3	18.0	18.3
		0.1	5	76.7	76.6	11.5	11.4
	Egg	0.01	5	91.4	91.9	4.1	4.7
		0.1	5	91.0	92.1	1.6*	2.6*

* without outlier

Linearity:	Good linearity was observed in the range between 0.10 and 5.0 ng/mL.
Specificity:	No interferences with sample matrices were reported.
Limit of Quantitation:	The limit of quantitation was defined by the lowest fortification level successfully tested which was 0.01 mg/kg for all examined matrices.
Repeatability:	The coefficients of variation with respect to recoveries following fortifications at the limit of quantitation were between 0.5% and 18.3% in all examined matrices. The values obtained are indicative of the method having satisfactory repeatability.
Reproducibility:	In this study, the reproducibility of the method was successfully proven by an independent laboratory validation.
Conclusion:	The independent lab validation approved the accurate determination of bentazone, 6-OH-bentazone and 8-OH-bentazone by using BASF Method No. 438/2 in whole milk, egg, bovine meat and liver specimen. The limit of quantitation was 0.01 mg/kg for all animal commodities tested. Therefore it is shown that BASF Method No. 438/2 fulfils all data requirements for enforcement and data generation.

4.4 Description of methods for analysis of soil (parent and metabolites)

Report:	II A 4.4/1 Penning H. 2009(c) Validation of analytical method L0136/01 for the LC-MS/MS determination of BAS 351 H (Bentazon) and its metabolite BH 351-N-Me (Reg.No. 79520) in soil and sediment BASF DocID 2009/1091211
Guidelines:	EEC 91/414 Annex II; EEC 91/414 Annex III; SANCO/825/00 rev. 7 (17 March 2004); SANCO/3029/99 rev. 4 (11 July 2000); EPA 850.7100
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the method

A 5 g soil or sediment sample is extracted with 50 mL methanol/water (50/50, v/v) by mechanical shaking for 60 min at 225 rpm. A 5 mL aliquot of the extract is centrifuged for 5 min at 4000 rpm (20°C). The extract is taken directly or diluted with methanol/water (50/50, v/v) to the appropriate final volume and measured by UPLC-MS/MS or HPLC-MS/MS.

Recovery findings

The method proved to be suitable to determine residues of BAS 351 H and its metabolite N-methyl-bentazone (Reg.No. 79520) in soil and sediment to a limit of quantification (LOQ) of 0.01 mg/kg by UPLC-MS/MS or HPLC-MS/MS. In the validation experiments, the mean recovery values were between 70% and 110%. The detailed results are given in Table 4.4/1 for HPLC-MS/MS and in Table 4.4/2 for UPLC-MS/MS.

Table 4.4/1 Validation results of method L0136/01: BAS 351 H and its metabolite N-methyl-bentazone in soil and sediment (HPLC-MS/MS)

Matrix	Test Item	Transition	Fortification Level [mg/kg]	Number of Replicates	Mean Recovery [%]	SD [%]	RSD [%]
LUFA 2.2 Soil	BAS 351 H	239 --> 132	0.01	5	101.2	0.6	0.6
			0.1	5	99.9	0.8	0.8
			1	5	100.2	0.5	0.5
		239 --> 197	0.01	5	101.0	1.3	1.2
			0.1	5	100.2	1.3	1.3
			1	5	100.7	1.3	1.3
	BH 351-N-Me	255 --> 134	0.01	5	96.2	1.4	1.5
			0.1	5	96.2	1.5	1.5
			1	5	97.6	1.4	1.4
		255 --> 213	0.01	5	96.3	1.3	1.3
			0.1	5	98.2	0.8	0.8
			1	5	98.8	0.8	0.8
LUFA 5M Soil	BAS 351 H	239 --> 132	0.01	5	101.9	2.5	2.5
			0.1	5	100.6	0.5	0.5
			1	5	99.1	2.4	2.4
		239 --> 197	0.01	5	101.8	1.4	1.3
			0.1	5	101.1	1.7	1.6
			1	5	100.4	2.4	2.3
	BH 351-N-Me	255 --> 134	0.01	5	94.7	1.0	1.0
			0.1	5	98.8	0.9	0.9
			1	5	99.2	1.1	1.1
		255 --> 213	0.01	5	93.8	0.5	0.5
			0.1	5	98.3	1.1	1.1
			1	5	98.4	0.7	0.7
Ransch-graben Sediment	BAS 351 H	239 --> 132	0.01	5	101.8	2.5	2.4
			0.1	5	97.8	0.8	0.9
			1	5	96.0	1.2	1.3
		239 --> 197	0.01	5	102.5	2.9	2.9
			0.1	5	98.1	1.0	1.0
			1	5	96.0	1.1	1.2
	BH 351-N-Me	255 --> 134	0.01	5	87.4	0.3	0.4
			0.1	5	92.5	2.4	2.6
			1	5	95.1	1.5	1.5
		255 --> 213	0.01	5	87.9	1.4	1.6
			0.1	5	92.4	0.9	1.0
			1	5	95.7	1.1	1.1

Table 4.4/2 Validation results of method L0136/01: BAS 351 H and its metabolite N-methyl-bentazone in soil and sediment (UPLC-MS/MS)

Matrix	Test Item	Transition	Fortification Level [mg/kg]	Number of Replicates	Mean Recovery [%]	SD [%]	RSD [%]
LUFA 2.2 Soil	BAS 351 H	239 --> 132	0.01	5	92.8	2.5	2.7
			0.1	5	96.4	3.4	3.6
			1	5	98.2	3.4	3.5
		239 --> 197	0.01	5	94.7	3.7	3.9
			0.1	5	96.6	2.2	2.3
			1	5	98.5	4.1	4.2
	BH 351-N-Me	255 --> 134	0.01	5	99.2	2.8	2.8
			0.1	5	97.2	3.9	4.0
			1	5	97.4	2.7	2.7
		255 --> 213	0.01	5	97.5	1.7	1.8
			0.1	5	98.3	2.8	2.9
			1	5	97.2	1.3	1.3
LUFA 5M Soil	BAS 351 H	239 --> 132	0.01	5	91.4	4.3	4.7
			0.1	5	96.6	1.8	1.8
			1	5	95.4	2.4	2.5
		239 --> 197	0.01	5	92.4	4.1	4.4
			0.1	5	97.1	1.8	1.8
			1	5	95.0	3.5	3.7
	BH 351-N-Me	255 --> 134	0.01	5	97.6	2.0	2.0
			0.1	5	100.6	2.4	2.4
			1	5	98.2	2.0	2.0
		255 --> 213	0.01	5	96.4	1.6	1.6
			0.1	5	100.1	3.1	3.1
			1	5	97.9	3.0	3.0
Ransch-graben Sediment	BAS 351 H	239 --> 132	0.01	5	97.6	1.9	2.0
			0.1	5	96.1	2.8	2.9
			1	5	95.8	2.9	3.0
		239 --> 197	0.01	5	96.8	2.0	2.0
			0.1	5	96.6	2.7	2.8
			1	5	95.8	3.3	3.4
	BH 351-N-Me	255 --> 134	0.01	5	95.8	1.2	1.2
			0.1	5	94.9	2.8	2.9
			1	5	96.7	2.0	2.0
		255 --> 213	0.01	5	101.1	2.4	2.4
			0.1	5	98.6	1.9	1.9
			1	5	95.7	2.5	2.6

Linearity

Good linearity was observed in the range of 0.005 ng/mL to 0.25 ng/mL for BAS 351 H and its metabolite N-methyl-bentazone ($r > 0.998$).

Specificity
The method determines BAS 351 H and its metabolite N-methyl-bentazone. A second mass transition was quantified per analyte demonstrating specificity. No significant interferences (< 30% of LOQ) were present at the retention time and mass transitions of BAS 351 H and N-methyl-bentazone. The blank values were below the LOD (< 10% LOQ) in soil and sediment for both mass transitions.

Limit of Quantification
The limit of quantification (LOQ) was defined by the lowest fortification level successfully tested, which was 0.01 mg/kg for both analytes.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.4/2.

Reproducibility
Reproducibility of the method was not determined within this validation study.

Conclusion
Method L0136/01 for analysis of BAS 351 H and its metabolite N-methyl-bentazone uses LC-MS/MS for final determination, which is a highly specific technique. The limit of quantification is 0.01 mg/kg for both analytes.

It could be demonstrated that the method fulfills the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of BAS 351 H and its metabolite N-methyl-bentazone in soil and sediment.

4.5 Description of methods of analysis of water (parent and metabolites)

Report: II A 4.5/1
Penning H. 2009(d)
Validation of analytical method L0141/01 for the LC-MS/MS determination of BAS 351 H (Bentazon) in surface water and groundwater
BASF DocID 2009/1076476

Guidelines: EEC 91/414 Annex IIA; EEC 91/414 Annex IIIA; SANCO/825/00 rev. 7 (17 March 2004); SANCO/3029/99 rev. 4 (11 July 2000); EPA 850.7100; EEC 96/46

GLP: Yes
(laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the method
A 10 g aliquot of the water sample is adjusted to pH 2 and extracted by SPE. The analyte is eluted with methanol. After evaporation to dryness the residues are dissolved in water/methanol (50 + 50, v + v). An aliquot of the final volume is measured using LC-MS/MS.

Recovery findings
The method proved to be suitable to determine residues of BAS 351 H in surface water and groundwater to a limit of quantification (LOQ) of 0.03 µg/kg by HPLC-MS/MS. In the validation experiments, the mean recovery values were between 70% and 110%. The detailed results are given in Table 4.5/1.

Table 4.5/1 Validation results of method L0141/01: BAS 351 H in surface water and groundwater

Test Item	Matrix	Transition	Fortification Level [µg/kg]	Number of Replicates	Mean Recovery [%]	SD [%]	RSD [%]
BAS 351 H	Ground-water	239 --> 132	0.03	4	94.0	6.8	7.3
			0.3	5	82.1	1.6	1.9
		239 --> 197	0.03	4	94.8	6.6	7.0
			0.3	5	82.8	1.5	1.8
	Surface Water	239 --> 132	0.03	5	77.5	5.1	6.6
			0.3	5	85.7	1.5	1.7
		239 --> 197	0.03	5	76.1	5.0	6.6
			0.3	5	85.3	1.7	2.0

Linearity
Good linearity was observed in the range of 0.025 ng/mL to 1.0 ng/mL for BAS 351 H ($r > 0.999$).

Specificity
The method determines BAS 351 H. A second mass transitions was quantified per analyte demonstrating specificity. No significant interferences (< 30% of LOQ) were present at the retention time and mass transitions of BAS 351 H. The blank values were below the LOD (< 17% LOQ) in surface water and groundwater for both mass transitions.

Limit of Quantification
The limit of quantification (LOQ) was defined by the lowest fortification level successfully tested, which was 0.03 µg/kg for BAS 351 H.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.5/1.

Reproducibility
Reproducibility of the method was not determined within this validation study.

Conclusion
Method L0141/01 for analysis of BAS 351 H uses HPLC-MS/MS for final determination, which is a highly specific technique. The limit of quantification is 0.03 µg/kg for BAS 351 H.

It could be demonstrated that the method fulfills the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of BAS 351 H in surface water and groundwater.

Dossier update September 2013

Report:	II A 4.5/2 Daum, A. 2004 Validation of the Method APL0430/01 Determination of BH 351-N-Me (RegNo 79520) in Water by HPLC BASF DocID 2004/1005046
Guidelines:	n.a.
GLP:	Yes (OECD Principles of Good Laboratory Practice), GLP Principles of the German “Chemikaliengesetzt” (Chemical Act).

Principle of the method
Samples are diluted into a concentration range within the calibration series, care being taken that the final dilution contains 30% acetonitrile. Subsequently, 10 µL aliquots of the sample are injected directly into the HPLC system for analysis.

Recovery findings
The method proved to be suitable to determine residues of BH 351-N-Me (RegNo. 79520) in water samples to a limit of quantification (LOQ) of 1 mg/L by HPLC-UV. In the validation experiments, the mean recovery values were between 97.7% and 101.3%.

Table 4.5/2 Validation results of method APL0430/01: BH 351-N-Me in test water, Frankenthaler (FT) water

Test Item	Matrix	UV wavelength (nm)	Fortification Level [mg/L]	Number of Replicates	Recoveries [%]
BAS 351-N-Me	FT water	216	1	5	97.7 – 98.8
			10	5	98.8 – 100.6
			50	5	99.5 – 101.3

Specificity

No relevant impurities were detected in the area of the test item peak. No interference was observed between the test substance with peaks from the test waters (no interference peaks in the blank samples).

Limit of Quantification

The limit of quantification (LOQ) was 1 mg/L.

Repeatability

The relative standard deviations (RSD, %) for all fortification levels were below 20%.

Reproducibility

Reproducibility of the method was not determined within this validation study.

Conclusion

Method APL0430/01 for analysis of BH 351-N-Me (Reg. No. 79520) uses HPLC-UV for final determination. The limit of quantification is 1 mg/L for BH 351-N-Me.

It could be demonstrated that the method fulfills the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of BH 351-N-Me in water.

4.6 Method for determining pesticides in sediment

Report:	II A 4.6/1 Penning H. 2009(c) Validation of analytical method L0136/01 for the LC-MS/MS determination of BAS 351 H (Bentazon) and its metabolite BH 351-N-Me (Reg.No. 79520) in soil and sediment BASF DocID 2009/1091211
Guidelines:	EEC 91/414 Annex II; EEC 91/414 Annex III; SANCO/825/00 rev. 7 (17 March 2004); SANCO/3029/99 rev. 4 (11 July 2000); EPA 850.7100
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the method

A 5 g soil or sediment sample is extracted with 50 mL methanol/water (50/50, v/v) by mechanical shaking for 60 min at 225 rpm. A 5 mL aliquot of the extract is centrifuged for 5 min at 4000 rpm (20°C). The extract is taken directly or diluted with methanol/water (50/50, v/v) to the appropriate final volume and measured by UPLC-MS/MS or HPLC-MS/MS. The analysis of a set of 17 samples requires about 1.5 days of work.

Recovery findings

The method proved to be suitable to determine residues of BAS 351 H and its metabolite BH 351-N-Me (Reg.No. 79520) in soil and sediment to a limit of quantification (LOQ) of 0.01 mg/kg by UPLC-MS/MS or HPLC-MS/MS. In the validation experiments, the mean recovery values were between 70% and 110%. The detailed results are given in Table 4.6/1 for HPLC-MS/MS and in Table 4.6/2 for UPLC-MS/MS.

Table 4.6/1 Validation results of method L0136/01: BAS 351 H and its metabolite BH 351 N-Me (Reg.No. 79520) in soil and sediment (HPLC-MS/MS)

Matrix	Test Item	Transition	Fortification Level [mg/kg]	Number of Replicates	Mean Recovery [%]	SD [%]	RSD [%]
LUFA 2.2 Soil	BAS 351 H	239 --> 132	0.01	5	101.2	0.6	0.6
			0.1	5	99.9	0.8	0.8
			1	5	100.2	0.5	0.5
		239 --> 197	0.01	5	101.0	1.3	1.2
			0.1	5	100.2	1.3	1.3
			1	5	100.7	1.3	1.3
	BH 351-N-Me	255 --> 134	0.01	5	96.2	1.4	1.5
			0.1	5	96.2	1.5	1.5
			1	5	97.6	1.4	1.4
		255 --> 213	0.01	5	96.3	1.3	1.3
			0.1	5	98.2	0.8	0.8
			1	5	98.8	0.8	0.8
LUFA 5M Soil	BAS 351 H	239 --> 132	0.01	5	101.9	2.5	2.5
			0.1	5	100.6	0.5	0.5
			1	5	99.1	2.4	2.4
		239 --> 197	0.01	5	101.8	1.4	1.3
			0.1	5	101.1	1.7	1.6
			1	5	100.4	2.4	2.3
	BH 351-N-Me	255 --> 134	0.01	5	94.7	1.0	1.0
			0.1	5	98.8	0.9	0.9
			1	5	99.2	1.1	1.1
		255 --> 213	0.01	5	93.8	0.5	0.5
			0.1	5	98.3	1.1	1.1
			1	5	98.4	0.7	0.7
Ransch-graben Sediment	BAS 351 H	239 --> 132	0.01	5	101.8	2.5	2.4
			0.1	5	97.8	0.8	0.9
			1	5	96.0	1.2	1.3
		239 --> 197	0.01	5	102.5	2.9	2.9
			0.1	5	98.1	1.0	1.0
			1	5	96.0	1.1	1.2
	BH 351-N-Me	255 --> 134	0.01	5	87.4	0.3	0.4
			0.1	5	92.5	2.4	2.6
			1	5	95.1	1.5	1.5
		255 --> 213	0.01	5	87.9	1.4	1.6
			0.1	5	92.4	0.9	1.0
			1	5	95.7	1.1	1.1

Table 4.6/2 Validation results of method L0136/01: BAS 351 H and its metabolite BH 351 N-Me (Reg.No. 79520) in soil and sediment (UPLC-MS/MS)

Matrix	Test Item	Transition	Fortification Level [mg/kg]	Number of Replicates	Mean Recovery [%]	SD [%]	RSD [%]
LUFA 2.2 Soil	BAS 351 H	239 --> 132	0.01	5	92.8	2.5	2.7
			0.1	5	96.4	3.4	3.6
			1	5	98.2	3.4	3.5
		239 --> 197	0.01	5	94.7	3.7	3.9
			0.1	5	96.6	2.2	2.3
			1	5	98.5	4.1	4.2
	BH 351-N-Me	255 --> 134	0.01	5	99.2	2.8	2.8
			0.1	5	97.2	3.9	4.0
			1	5	97.4	2.7	2.7
		255 --> 213	0.01	5	97.5	1.7	1.8
			0.1	5	98.3	2.8	2.9
			1	5	97.2	1.3	1.3
LUFA 5M Soil	BAS 351 H	239 --> 132	0.01	5	91.4	4.3	4.7
			0.1	5	96.6	1.8	1.8
			1	5	95.4	2.4	2.5
		239 --> 197	0.01	5	92.4	4.1	4.4
			0.1	5	97.1	1.8	1.8
			1	5	95.0	3.5	3.7
	BH 351-N-Me	255 --> 134	0.01	5	97.6	2.0	2.0
			0.1	5	100.6	2.4	2.4
			1	5	98.2	2.0	2.0
		255 --> 213	0.01	5	96.4	1.6	1.6
			0.1	5	100.1	3.1	3.1
			1	5	97.9	3.0	3.0
Ransch-graben Sediment	BAS 351 H	239 --> 132	0.01	5	97.6	1.9	2.0
			0.1	5	96.1	2.8	2.9
			1	5	95.8	2.9	3.0
		239 --> 197	0.01	5	96.8	2.0	2.0
			0.1	5	96.6	2.7	2.8
			1	5	95.8	3.3	3.4
	BH 351-N-Me	255 --> 134	0.01	5	95.8	1.2	1.2
			0.1	5	94.9	2.8	2.9
			1	5	96.7	2.0	2.0
		255 --> 213	0.01	5	101.1	2.4	2.4
			0.1	5	98.6	1.9	1.9
			1	5	95.7	2.5	2.6

Linearity
Good linearity was observed in the range of 0.005 ng/mL to 0.25 ng/mL for BAS 351 H and its metabolite BH 351 N-Me (Reg.No. 79520) ($r > 0.998$).

Specificity
The method determines BAS 351 H and its metabolite BH 351 N-Me (Reg.No. 79520).
A second mass transitions was quantified per analyte demonstrating specificity. No significant interferences (< 30% of LOQ) were present at the retention time and mass transitions of BAS 351 H and BH 351-N-Me. The blank values were below the LOD (< 10% LOQ) in soil and sediment for both mass transitions.

Limit of Quantification
The limit of quantification (LOQ) was defined by the lowest fortification level successfully tested, which was 0.01 mg/kg for both analytes.

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 5.4-2.

Reproducibility
Reproducibility of the method was not determined within this validation study.

Conclusion
Method L0136/01 for analysis of BAS 351 H and its metabolite BH 351 N-Me (Reg.No. 79520) uses LC-MS/MS for final determination, which is a highly specific technique. The limit of quantification is 0.01 mg/kg for both analytes.

It could be demonstrated that the method fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of BAS 351 H and its metabolite BH 351 N-Me (Reg.No. 79520) in soil and sediment.

4.7 Methods for analysis of air (parent and metabolites)

Report: II A 4.7/1
Penning H. 2010(b)
Validation of analytical method L0162/01: Determination of BAS 351 H (Bentazon) in air by HPLC-MS/MS
BASF DocID 2010/1110705

Guidelines: SANCO/825/00 rev. 7 (17 March 2004); SANCO/3029/99 rev. 4 (11 July 2000)

GLP: Yes
(laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Principle of the method
The analyte is spiked to Tenax adsorption tubes. After sucking air through the glass tubes for about 6 hours, adsorbers are extracted by ultrasonication using acetone. An aliquot of the extract is reduced to dryness and reconstituted in methanol/water (50/50, v/v). Final determination of BAS 351 H is achieved by HPLC-MS/MS.

Test conditions
The relative humidity was above 80% at a temperature of 35 °C. The collected air volume was 540 L with a sampling rate of 90 L / h for 6 hours.

Recovery findings
The method proved to be suitable to determine residues of BAS 351 H in air to a limit of quantification (LOQ) of 0.37 ng BAS 351 H per L air (0.01 C). The mean recovery values of the validation experiments were between 70% and 110%. The detailed results are given in Table 4.7/1.

Table 4.7/1 Validation results of method L0162/01: Bentazone (BAS 351 H) in air

Test Item	Matrix	Transition	Fortification Level	Number of Replicates	Mean Recovery [%]	SD [%]	RSD [%]
BAS 351 H	Air	239 --> 132	0.01 C	6	81	6.5	8.1
			0.1 C	6	90	3.6	4.0
		239 --> 197	0.01 C	6	79	7.1	8.9
			0.1 C	6	89	2.9	3.3

Linearity
Good linearity ($r > 0.99$) was observed in the range of 0.025 ng/mL to 0.5 ng/mL for both mass transitions of BAS 351 H.

Specificity
The method determines BAS 351 H in air. Significant interferences (> 30% of LOQ) were not observed at the retention time and mass transitions of BAS 351 H. Due to the high selectivity and specificity of LC-MS/MS, an additional confirmatory technique is not necessary. Two mass transitions of BAS 351 H were quantified.

Limit of Quantification
The limit of quantification (LOQ) was defined by the lowest fortification level successfully tested corresponding to a concentration of 0.37 ng BAS 351 H per L air (0.01 C).

Repeatability
The relative standard deviations (RSD, %) for all fortification levels were below 20%. The detailed values are shown in Table 4.7/1.

Reproducibility
Reproducibility of the method was not determined within this validation study.

Conclusion
It could be demonstrated that method L0162/01 fulfils the requirements with regard to specificity, repeatability, limit of quantification and recoveries and is therefore applicable to correctly determine residues of BAS 351 H in air.

4.8 Methods for analysis of body fluid/tissues (parent and metabolites)

An analytical method for residues in body fluids and tissues is not required as bentazone is neither toxic nor very toxic (classification: Xn).

4.9 Other/special studies

No other/special studies were performed.

BASF
The Chemical Company

DOSSIER FOR THE EVALUATION OF THE ACTIVE SUBSTANCE

Bentazone (BAS 351 H)

Data requirement according to Regulation (EU) No. 544/2011 and (EU) No. 545/2011 (formerly Annex II and III of Directive 91/414/EEC) using OECD Guidance for Industry Data Submissions on Plant Protection Products and their Active Substance (Dossier Guidance)

Document M-II

Summary and evaluation (Tier II)

Section 3

Toxicological and metabolism studies on the active substance

BASF DocID 2012/1007333

compiled by

[redacted]

Date: 24 February 2012

5 Toxicological and Toxicokinetic Studies on the Active Substance

List of Contents

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According to Article 1(c) of Regulation (EU) No. 1141/2010 the supplementary dossier includes data and risk assessments which were not part of the original dossier and which are necessary to reflect changes. Reasoning for each test or study as required by Art 1(d)/(e) of the Regulation is given in the reference list of the dossier.

According to Article 1(f) of Regulation (EU) No. 1141/2010 the supplementary dossier shall include a description of the steps taken to avoid animal testing and duplication of tests and studies on vertebrate animals for each test or study involving vertebrate animals.

All vertebrate studies have been conducted in order to fulfil the data requirements for the re-approval of the active substance and the authorisation of plant protection products. Furthermore, the test strategy was based on corresponding guidance documents takes into account methods compliant with the 3R concept for refinement, reduction and replacement of animal testing where applicable and acceptable.

In order to avoid duplication of tests and studies BASF has submitted a list of studies to be generated within the application. This information has been accessible to other co-notifiers.
Vice versa BASF had access to the respective information as provided by other co-notifiers.
In addition BASF has performed a literature search.
However, generally it has been assumed that BASF as the main data owner for bentazone will be contacted by co-notifiers before initiating vertebrate studies.

For Bentazone a literature search has been performed. The search report including a description of the selection and assessment process is provided in BASF DocID 2012/1007281 (DocLIIA).
The results of the selection process and assessments for Toxicological and metabolism studies are outlined in BASF DocID 2012/1007283.

5.1 Absorption, distribution, excretion and metabolism in mammals

The absorption, distribution, excretion and metabolism of bentazone (BAS 351 H) in rats were investigated using the active substance radiolabelled in the phenyl ring. The test item was a mixture of ^{14}C-bentazone (Phenyl Label) and unlabelled bentazone. The molecular structure and the position of the label are shown below:

Bentazone (BAS 351 H)
= Reg. No. 51929
= CAS No. 25057-89-0

Figure 5.1/1 ^{14}C-labelled bentazone

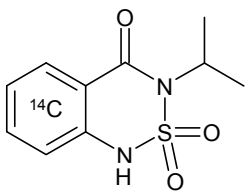
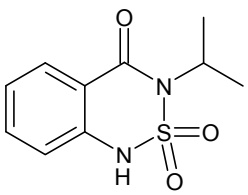


Figure 5.1/2 Unlabelled bentazone



Studies presented in the original Annex II Dossier (1995): A series of studies has been presented on rats, mice and rabbits to determine the kinetics of bentazone. The metabolic pathway of bentazone was elucidated in these species following oral (rat and rabbit) or intravenous administration (mouse).
These studies have been evaluated by European authorities and Germany as Rapporteur member state in 1998 (European Commission Peer Review Program, BASF Doc ID 1998/1001178) and the endpoints were fixed.
Studies on toxicokinetics have shown a high bioavailability of bentazone following oral administration and low bioavailability of less than 2% of total dose after dermal administration. Elimination has been shown to be rapid with urine being the major route of excretion (approximately 90% of the dose). Only traces (1% to 2 %) were found in the feces and in the bile. Repeated administration did not significantly alter the pattern of absorption and elimination. There was no evidence for accumulation of bentazone but an indication of a non-linear region of plasma levels over time. In all species investigated (rat, rabbit. mouse, goat and hen), kinetic studies revealed considerable similarities.
Bentazone was only poorly metabolized with the parent compound being the predominant excretion product. Only small amounts of the metabolites 6-OH-bentazone and 8-OH-bentazone could be detected. In rats, rabbits and mice, no conjugated products were found. However, conjugation did occur in goat and hen.

Submission of not yet peer-reviewed studies in this AIR2 Dossier:
A verification of the metabolite identification has been performed since more sophisticated analytical technology is available. This study confirmed the older studies. Regarding the possibility of a non-linear region of plasma levels over time, a plasma kinetic study and a mechanistic study to elucidate the elimination path of Bentazone were performed in female Wistar rats. These studies show that Bentazone is actively secreted via the saturable renal Organic Anion Transporter, and saturation of excretion starts between actual dose levels of 84.7 and 165.9 mg/kg bw (calculated as Bentazone sodium salt).
Additionally, the dermal absorption of Bentazone has been investigated in the representative SL-type solo formulation BAS 351 32 H in vitro in human skin and revealed a maximum absorption of 1.3 % of the total dose (see AIR 2-Dossier Document M-III A 07.06), thereby confirming the low dermal bioavailability of Bentazone.

5.1.1 Toxicokinetic studies - Single dose, oral route, in rats

Report:	II A 5.1.1/1 [redacted] 2011(c) Excretion and metabolism of 14C-Bentazone (BAS 351 H, Reg.No. 51929) after oral administration in rats BASF DocID 2010/1027274
Date of report:	07-Apr-2011
Testing facility:	[redacted]
Guidelines:	EPA 870.7485; EPA 860.1000; OECD 417; EEC 87/302 B; MAFF Testing Guidelines for Toxicology Studies: Metabolism Animals (Japan)
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	II A 5.1.1/2 [redacted] 2011(d) Amendment No.1 - Excretion and metabolism of 14C-Bentazone (BAS 351 H, Reg.No. 51929) after oral administration in rats BASF DocID 2011/1197846
Date of report:	08-Aug-2011
Testing facility:	[redacted]
Guidelines:	EPA 870.7485; EPA 860.1000; OECD 417; EEC 87/302 B; MAFF Testing Guidelines for Toxicology Studies: Metabolism Animals (Japan)
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The excretion and metabolism of BAS 351 H was investigated in male and female rats after application of a single oral nominal dose of 200 mg/kg bodyweight. To facilitate metabolite identification by mass spectroscopy and HPLC, a mixture of ^{14}C-labelled and unlabelled test substance was applied. Urine and faeces were collected after 6, 12, 24 hours and thereafter in intervals of 24 hours for a total of 7 days.
The excretion and metabolism was similar in both sexes. Excretion was nearly complete in 72 hours and the majority of the dose was excreted via urine in both male and female rats (65.08% and 65.01%, respectively). Smaller portions of the dose were excreted via faeces at comparable levels for males and females (3.20% and 2.22%, respectively). The cage wash accounted for 11.31% and 9.14% of the dose while only minor amounts of radioactivity were found in organs and animal remains (0.41% and 0.40% of the dose, respectively).

In urine, the metabolic pattern was similar in male and female rats. Besides unchanged BAS 351 H (males: 62.70% and females: 63.97% of the dose), small amounts of metabolite M351H001 were excreted in urine (males: 3.15% and females: 0.52% of the dose). Metabolite M351H001 resulted from hydroxylation of the parent compound. Further urine metabolites identified in males and females by mass spectroscopy were present only in trace amounts (M351H002, M351H003, M351H004, M351H005, M351H007 and M351H008).
In faeces, the metabolic pattern was also similar in male and female rats. Besides unchanged BAS 351 H (males: 0.86% and females: 0.68% of the dose), small amounts of metabolites M351H001 and M351H002 were excreted. Metabolite M351H002 resulted from hydroxylation of the parent compound and is an isomer of M351H001. Metabolite M351H001 accounted for 0.42% (males) and 0.05% (females) of the dose, whereas 0.10% (males) and 0.05% (females) were excreted via faeces as metabolite M351H002. Trace amounts of metabolite M351H006 were detected by mass spectroscopy in faeces of male rats.
The two metabolites M351H001 and M351H002, which were excreted at quantifiable levels, resulted from hydroxylation of the parent compound at position six of the benzothiadiazine ring and position eight, respectively. Further metabolites, which were present only in trace amounts, resulted from glucuronidation of metabolite M351H001 (M351H003), N-glucuronidation (M351H004), dealkylation (M351H005), hydroxylation (M351H006), N-glucosylation (M351H007) and dehydrogenation (M351H008) of the parent compound.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Bentazone
   Description: ^{14}C in the Phenyl ring ("Phenyl label"; Phenyl-U-^{14}C), specific activity a.s. 5.15 MBq/mg (309000 dpm/µg); unlabelled bentazone
   Chemical Name: 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxid
   BASF Registry No.: 51929
   Batch No.: 210-2301 (14C-labelled), 01893-210 (unlabelled)
   Purity: Radiochemical purity of labelled test item: 96.5%
   Chemical purity of unlabelled test item: 99.8%
   Stability of test compound:
   To demonstrate the stability of the test item in the application formulation, HPLC analysis of the application formulation was performed.

2. **Vehicle and/or positive control:**
   0.5% aqueous solution of carboxymethyl cellulose containing 1% of cremophor

3. **Test animals:**
   Species: Rat
   Strain: Wistar Crl: WI (Han)
   Age: Not reported
   Weight at dosing: Average of 271 g for males and 244 g for females
   Source: Charles River Laboratories, Sulzfeld, Germany
   Diet: Kliba lab diet for rat-mouse-hamster ("GLP", Provimi Kliba SA, Kaiseraugst, Switzerland). *Ad libitum*.
   Water: Tap water *ad libitum*
   Housing: During acclimatisation in macrolon cages. During experiments in Plexiglas metabolism cages.
   Environmental conditions:
   Temperature: 21 - 24°C
   Humidity: 55 - 75%
   Air change: Not reported
   Photoperiod: Day / night rhythm of 12 h

4. Preparation of dosing solutions

The ^{14}C-labelled test item dissolved in acetonitrile was mixed with the unlabelled test item in such a way that after subsequent evaporation of the solvent a specific activity of about 15000 dpm/pg was achieved. Thereafter, the mixture of ^{14}C-labelled and unlabelled test item was resuspended in 0.5% aqueous solution of carboxymethyl cellulose containing 1% of cremophor. Prior to administration, the mixture was sonicated and stirred to produce a homogenous preparation. The application solution was liquid scintillation counting (LSC) measured and the measured value was taken for calculation of the concentration of the test item. Furthermore, to demonstrate the stability of the test item in the application formulation, HPLC analysis of the application formulation was performed. The single oral doses were weighed into syringes and administered orally by gavage. The amount of test item actually applied was calculated by weighing of the empty syringe.

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 07-Sep-2009 - 06-Jul-2010

2. Study design

The study was designed to obtain data on the rates and routes of excretion of bentazone after oral administration of the test item and to investigate the nature of the bentazone biotransformation products in excreta of rats.
Four male and four female rats were dosed once orally with a mixture of ^{14}C-labelled and unlabelled bentazone at a nominal dose level of 200 mg/kg bodyweight.

Sampling and storage
Urine and faeces were sampled at 6, 12 and 24 hours post application and thereafter in 24 hour intervals, until 168 hours. Samples were pooled for males and females separately. No faeces were collected from female rats at 6 hours, as there was no defecation in the respective time period. The animals were sacrificed in a carbon dioxide chamber seven days after dosing and the metabolism cages were subsequently washed. All the samples (urine, faeces, carcasses and cage wash) were frozen and stored at -18°C prior to analysis.

Determination of radioactivity
For the quantitation of radioactivity in liquid samples, a liquid scintillation counter was used. Aliquots (typically three) of liquid samples were mixed with a sufficient volume of a suitable scintillator prior to measurement and analysed for radioactivity without any additional treatment. All data were corrected using appropriate quench curves and are expressed in decays per minute (dpm).
In case of solid samples, aliquots of the homogenised samples were weighed into combustion cones and combusted by means of an automatic sample oxidiser. The $^{14}CO_2$ formed during combustion was trapped by an absorption liquid and the collected radioactivity was measured by LSC. Measurements of radioactivity were corrected for oxidiser efficiency.

HPLC analysis
For the determination of the metabolic patterns, metabolite identification and quantitation of metabolites, two different reversed phase HPLC methods were applied. Different stationary phases with gradient elution were used for metabolite profiling, quantitation and identification of metabolites.

Sample preparation for analysis

In order to investigate the metabolic patterns, appropriate aliquots of the urine samples from males and females (all time intervals) were directly subjected to radio-HPLC. Additionally, urine pool samples of male and female rats (time interval 0-72 h) containing 2% of the individual time intervals (0-6 h, 6-12 h, 12-24 h, 24-48 h and 48-72 h) were investigated using the HPLC methods LC01 and LC03.

For identification of metabolites in urine of male and female rats, samples of time interval 6-12 h were subjected to HPLC-MS analysis without any further sample preparation. Additionally, an aliquot of the urine sample of male rats 6-12 h was purified over a SPE column, whereby elution was carried out with methanol, and analysed by HPLC-MS.

The work-up procedures of faeces of male rats (12-24 h, 24-48 h and 48-72 h) and faeces of female rats (12-24 h and 24-48 h) are described below. Due to the low radioactivity in the other faeces samples (<0.25% of the dose), faeces prior to or after these time intervals were not extracted.

Homogenised faeces aliquots of male and female rats were extracted three times for 5 min with appropriate volumes of methanol using an ultra turrax at about 8000 rpm. The extracts were centrifuged, adjusted to defined volumes, LSC measured and pooled. Thereafter, the residues were extracted two times with water following the same extraction protocol. The water extracts were also LSC measured and pooled. The methanol extracts were directly subjected to radio-HPLC, whereas the water extracts were concentrated using a rotary evaporator at 40°C, dissolved in water and centrifuged prior to analysis. The extracts after solvent extraction were resuspended in approximately 30 mL Tris buffer (pH 7.0) and incubated at 37°C under shaking conditions. Then, two aliquots of about 10 mg protease per 1 g sample were added to the mixture (the first aliquot when the mixture was at 37°C and the second after 3.5 h) and incubation was carried out for 24 h. Thereafter, the mixture was centrifuged and the supernatant LSC measured. The residue was dried under a fume hood.

For identification of metabolites in faeces of male and female rats, the methanol extracts of time interval 12-24 h were concentrated using a rotary evaporator at 40°C, dissolved in methanol, centrifuged and subjected to HPLC-MS analysis.

Determination of the total radioactive residue (TRR)
Total radioactivity in urine samples and in the cage wash was determined by direct LSC.
Aliquots of faeces were homogenised by means of an analytical mill or a spatula prior to LSC. For determination of the total radioactivity in liver, kidney and animal remains the carcasses were thawn and livers and kidneys were removed. The samples were homogenised with the aid of a blender (liver), scalpels (kidney) and a meat grinder (animal remains).
The TRR was used as basis for calculation of extractability and of absolute quantities of metabolites.

II. RESULTS AND DISCUSSION

1. Excretion and distribution of radioactivity

A summary of the excretion balance is shown in Table 5.1/1. In both male and female rats, the vast majority of radioactivity was excreted via urine (66.08% and 65.01% of the dose, respectively). Overall, excretion was fast and nearly completed after 72 hours. The slightly delayed excretion via urine in male rats (males: maximum of 20.10% for time interval 6-12 h versus females: maximum of 25.06% for time interval 0-6 h) was most likely due to the low urination rate of the male rats during time interval 0-6 h (males: 1.9 mL urine versus females: 6.3 mL). Only a minor part of the radioactivity was excreted via faeces, accounting for 3.20% of the dose in male rats and 2.22% in female rats. The cage wash of male and female rats contained 11.31% and 9.14% of the dose, respectively. Only minor amounts of radioactivity were found in organs and animal remains accounting for 0.41% and 0.40% of the dose (male and female rats, respectively).

Table 5.1/1 Excretion balance after oral administration of ^{14}C-bentazone

	Males	Females
	[% of the dose]	[% of the dose]
Urine 0-6 h[1)]	7.52	25.06
Urine 6-12 h[1)]	20.10	5.51
Urine 12-24 h	16.59	13.24
Urine 24-48 h	8.21	12.79
Urine 48-72 h	9.61	3.82
Urine 72-96 h	1.86	1.50
Urine 96-120 h	1.15	1.16
Urine 120-144 h	0.60	1.03
Urine 144-168 h	0.45	0.90
Total urine 0-168 h	**66.08**	**65.01**
Faeces 0-6 h	0.01	-[2)]
Faeces 6-12 h	0.02	0.07
Faeces 12-24 h	1.22	0.83
Faeces 12-48 h	0.63	0.83
Faeces 48-72 h	0.71	0.24
Faeces 72-96 h	0.20	0.05
Faeces 96-120 h	0.09	0.05
Faeces 120-144 h	0.12	0.10
Faeces 144-168 h	0.20	0.06
Total faeces 0-168 h	**3.20**	**2.22**
Total excreted	**69.28**	**67.23**
Liver	0.003	0.002
Kidney	0.002	0.001
Animal remains	0.401	0.398
Cage wash	11.311	9.142
Total	**81.00**	**76.77**

1) The slightly delayed excretion via urine in male rats was probably due to the low urination rate of the male rats during time interval 0-6 h (males: 1.9 mL urine vs. females: 6.3 mL)
2) No defecation in the respective time period

2. Extractability

There was no major difference in the extractability between males and females, using methanol and water as extraction solvent. The extractability with methanol was comparable for the different time intervals, although, as a general tendency, the higher the concentration of the sample was, the more efficient was the extraction. The extractability with water showed no differences for the different time intervals. The overall extractability with methanol was between 51.8% (males, 48-72 h) and 74.4% of the TRR (males, 12-24 h). The following extraction with water released between 16.2% (males, 12-24 h) and 21.0% of the TRR (females, 24-48 h).
The remaining radioactive residues after solvent extraction were still between 11.1% (males, 12-24 h) and 27.4% of the TRR (females, 24-48 h), for which reason the residues were solubilised with protease. Solubilisation released another 4.5% (males, 12-24 h) to 9.0% of the TRR (females, 24-48 h). The final residues after solvent extraction and protease solubilisation were between 8.8% (males, 12-24 h) and 21.6% of the TRR (females, 24-48 h), what corresponded to 0.11% and 0.18% of the dose, respectively.

3. Metabolites in urine

The composition of radioactivity in urine samples of male rats of individual time intervals is depicted in Table 5.1/2. For all time intervals the unchanged parent compound represented the main component (from 0.45% to 18.60% of the dose). Metabolite M351H001 was significantly less abundant than the parent compound (from not detectable to 1.49% of the dose). Up to two not identified peaks were present (each below or equal to 0.10% of the dose). Overall in male rats, 62.70% of the dose was excreted via urine as the parent compound BAS 351 H and 3.15% as metabolite M351H001. A portion of 65.84% of the dose was thus identified and an additional 0.24% of the dose was characterised by their chromatographic properties.
The composition of radioactivity in urine samples of female rats of individual time intervals is depicted in Table 5.1/3. For all time intervals the unchanged parent compound represented the main component (from 0.90% to 24.63% of the dose). Metabolite M351H001 was significantly less abundant than the parent compound (from not detectable to 0.29% of the dose). Up to three not identified peaks were present (each below or equal to 0.14% of the dose). In female rats, 63.97% of the dose was thus excreted via urine as the parent compound BAS 351 H, what was comparable to male rats. However, the amount of metabolite M351H001 was somewhat below the amount of the same metabolite in urine of males (male: 3.15% versus female: 0.52% of the dose). Overall in female rats, a portion of 64.49% of the dose was identified and an additional 0.51% of the dose was characterised by their chromatographic properties.

Table 5.1/2 Composition of radioactivity in urine of male rats after oral administration of ^{14}C-bentazone

	Composition of Radioactivity in % of Applied Dose									
Time Interval [h]	**0-6**	**6-12**	**12-24**	**24-48**	**48-72**	**72-96**	**96-120**	**120-144**	**144-168**	**Sum**
Identified										
BAS 351 H	7.15	18.60	15.93	7.79	9.21	1.81	1.15	0.60	0.45	62.70
M351H001	0.37	1.49	0.66	0.33	0.26	0.03	n.d.	n.d.	n.d.	3.15
Total identified	**7.52**	**20.10**	**16.59**	**8.13**	**9.47**	**1.84**	**1.15**	**0.60**	**0.45**	**65.84**
Characterised by HPLC										
Up to two peaks (each below or equal to 0.10% dose)	n.d.	n.d.	n.d.	0.08	0.14	0.01	n.d.	n.d.	n.d.	0.24
Total identified and characterised	**7.52**	**20.10**	**16.59**	**8.21**	**9.61**	**1.86**	**1.15**	**0.60**	**0.45**	**66.08**

Table 5.1/3 Composition of radioactivity in urine of female rats after oral administration of ^{14}C-bentazone

	Composition of Radioactivity in % of Applied Dose									
Time Interval [h]	**0-6**	**6-12**	**12-24**	**24-48**	**48-72**	**72-96**	**96-120**	**120-144**	**144-168**	**Sum**
Identified										
BAS 351 H	24.63	5.40	13.24	12.40	3.71	1.50	1.16	1.03	0.90	63.97
M351H001	0.29	0.03	n.d.	0.17	0.03	n.d.	n.d.	n.d.	n.d.	0.52
Total identified	**24.93**	**5.43**	**13.24**	**12.57**	**3.73**	**1.50**	**1.16**	**1.03**	**0.90**	**64.49**
Characterised by HPLC										
Up to three peaks (each below or equal to 0.14% dose)	0.13	0.08	n.d.	0.22	0.08	n.d.	n.d.	n.d.	n.d.	0.51
Total identified and characterised	**25.06**	**5.51**	**13.24**	**12.79**	**3.82**	**1.50**	**1.16**	**1.03**	**0.90**	**65.01**

4. Metabolites in faeces

The composition of radioactivity in faeces methanol extracts of male rats of the three analysed time intervals is depicted in Table 5.1/4. For all analysed time intervals the unchanged parent compound represented the main component (0.52%, 0.17% and 0.18% of the dose). Metabolite M351H001 was less abundant than the parent compound for all time intervals (0.20%, 0.14% and 0.08% of the dose). Metabolite M351H002 was significantly less abundant than the parent compound (from not detectable to 0.06% of the dose). Up to four not identified peaks were present (each below or equal to 0.13% of the dose). The composition of radioactivity in faeces water extracts of males of the three analysed time intervals is also depicted in Table 5.1/4. Analysis of all three time intervals resulted in series of peaks of different polarity, which were not identified but characterised by their chromatographic properties (up to 39 peaks, each below or equal to 0.03% of the dose). Overall, 0.86% of the dose was excreted via faeces as the parent compound BAS 351 H, 0.42% as metabolite M351H001 and 0.10% as metabolite M351H002. Portions of 0.29% of the dose (methanol extract) and 0.24% (water extract) were characterised by HPLC.
The composition of radioactivity in faeces methanol extracts of female rats of the two analysed time intervals is depicted in Table 5.1/5. For all analysed time intervals the unchanged parent compound represented the main component (0.43% and 0.25% of the dose). Metabolite M351H001 was significantly less abundant than the parent compound (0.05% of the dose, detected only for time interval 12-24 h). The amount of metabolite M351H001 was somewhat below the amount of the same metabolite in faeces methanol extract of males (male: 0.42% versus female: 0.05% of the dose). Metabolite M351H002 was also significantly less abundant than the parent compound (0.05% of the dose, detected only for time interval 12-24 h). Up to three not identified peaks were present (each below or equal to 0.08% of the dose). The composition of radioactivity in faeces water extracts of female rats of the two analysed time intervals is also depicted in Table 5.1/5. Analysis of the two time intervals resulted in series of peaks of different polarity, which were not identified but characterised by their chromatographic properties (up to 36 peaks, each below or equal to 0.03% of the dose). Overall, 0.68% of the dose was excreted via faeces as the parent compound BAS 351 H, 0.05% as metabolite M351H001 and 0.05% as metabolite M351H002. Portions of 0.22% of the dose (methanol extract) and 0.23% (water extract) were characterised by HPLC.

Table 5.1/4 Composition of radioactivity in faeces of male rats after oral administration of ^{14}C-bentazone

	Composition of Radioactivity in % of Applied Dose			
Time Interval [h]	**12-24**	**24-48**	**48-72**	**Sum**
Identified	**Faeces Methanol Extract**			
BAS 351 H	0.52	0.17	0.18	0.86
M351H001	0.20	0.14	0.08	0.42
M351H002	0.06	0.03	n.d.	0.10
Total identified	**0.78**	**0.34**	**0.26**	**1.38**
Characterised by HPLC				
Up to four peaks (each below or equal to 0.13% dose)	0.13	0.05	0.11	0.29
Total identified and characterised	**0.91**	**0.39**	**0.37**	**1.67**
Characterised by HPLC	**Faeces Water Extract**			
Up to 39 peaks (each below or equal to 0.03% dose)	0.10	0.07	0.07	0.24

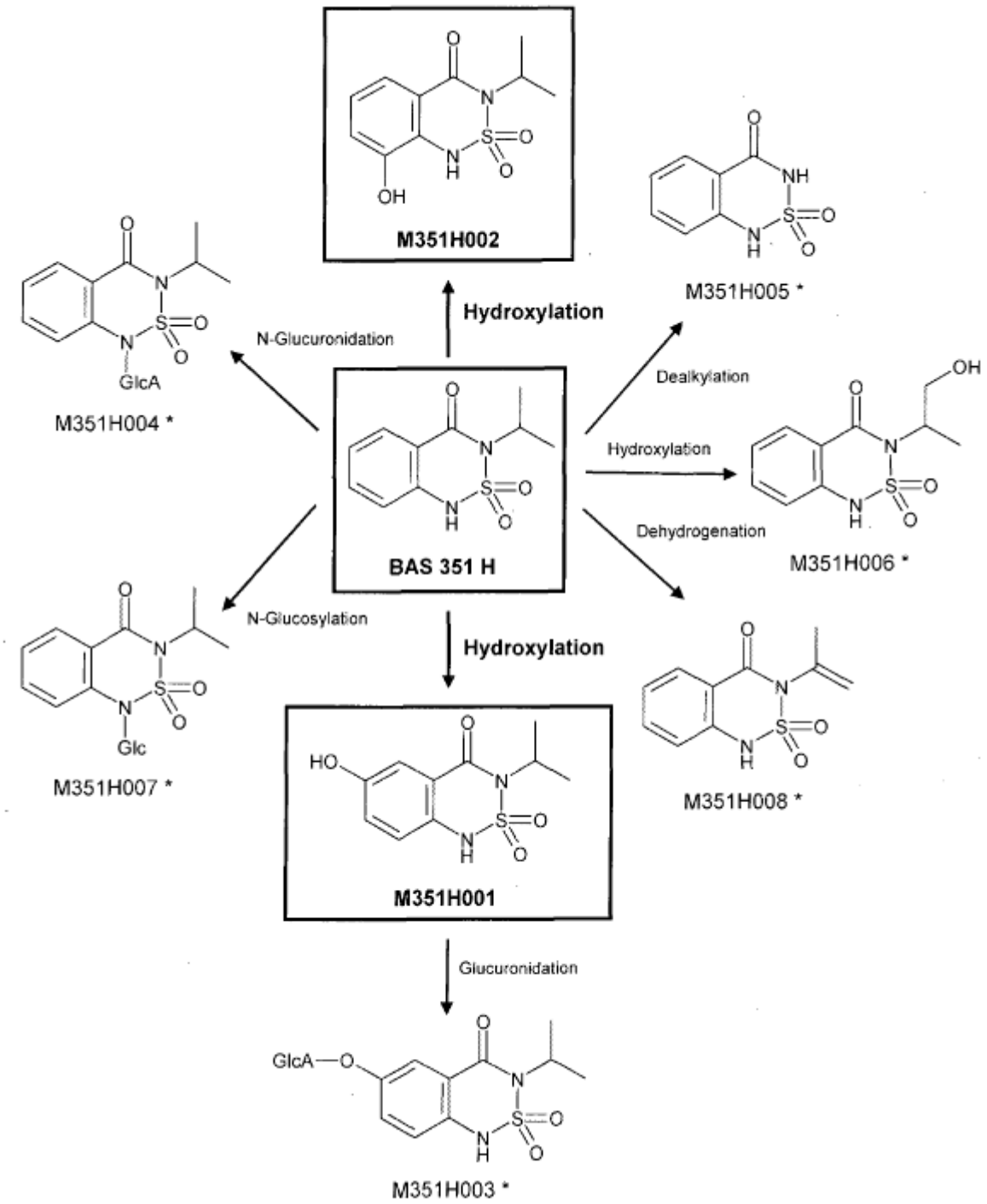
Table 5.1/5 Composition of radioactivity in faeces of female rats after oral administration of ^{14}C-bentazone

	Composition of Radioactivity in % of Applied Dose		
Time Interval [h]	**12-24**	**24-48**	**Sum**
Identified	**Faeces Methanol Extract**		
BAS 351 H	0.43	0.25	0.68
M351H001	0.05	n.d.	0.05
M351H002	0.05	n.d.	0.05
Total identified	**0.53**	**0.25**	**0.78**
Characterised by HPLC			
Up to three peaks (each below or equal to 0.08% dose)	0.03	0.19	0.22
Total identified and characterised	**0.56**	**0.44**	**1.01**
Characterised by HPLC	**Faeces Water Extract**		
Up to 36 peaks (each below or equal to 0.03% dose)	0.11	0.12	0.23

5. Metabolic Pathway

The overall metabolic pathway is depicted in Figure 5.1/3. All metabolites identified in the course of this study are shown. The major transformation steps in the pathway are hydroxylation of the benzothiadiazine ring at position six and eight.
The major part of BAS 351 H was excreted via urine as the unchanged parent compound. The two metabolites present in sufficient amounts for quantification resulted from hydroxylation of BAS 351 H at position six (M351H001) and position eight (M351H002). Further metabolites were present only in trace amounts: M351H003 resulted from glucuronidation of the hydroxyl group of metabolite M351H001 and M351H004 from glucuronidation of the nitrogen at position one. Dealkylation of the parent compound BAS 351 H led to metabolite M351H005. Metabolite M351H006 was formed by hydroxylation of one primary carbon atom of the parent compound. N-glucosylation of BAS 351 H resulted in metabolite M351H007 and dehydrogenation of the alkyl moiety of the parent compound in metabolite M351H008.

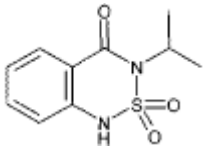
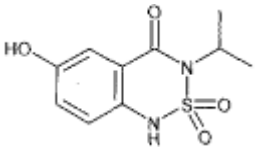
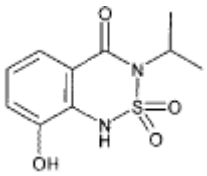
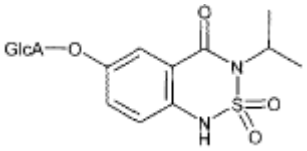
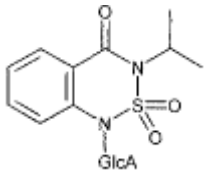
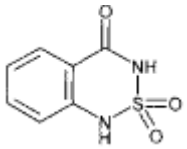
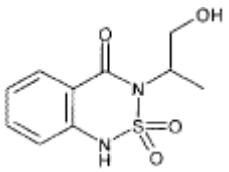
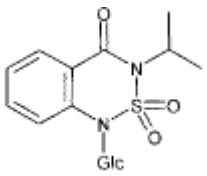
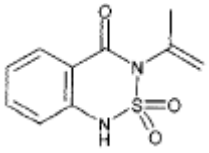
Figure 5.1/3 Proposed metabolic pathway of bentazone in rats



* Metabolites were identified by HPLC-MS. Identification and quantitation with HPLC was not feasible due to negligible amounts.

The following Table 5.1/6 summarises the metabolites of bentazone (BAS 351 H) identified in urine and faeces of rats.

Table 5.1/6 Summary of identified metabolites of bentazone in urine and faeces of rats

Metabolite Designation (Code)	Molecular Mass	Structure	Urine	Faeces
BAS 351 H (M351H000)	M = 240		X	X
M351H001	M = 256		X	X
M351H002	M = 256		X	X
M351H003	M = 432		X	-
M351H004	M = 416		X	-
M351H005	M = 197		X	-
M351H006	M = 256		-	X
M351H007	M = 402		X	-
M351H008	M = 238		X	-

III. CONCLUSION

Putting all data together, it can be concluded that bentazone (BAS 351 H) was metabolised to a rather small extent after a single oral dose application and was primarily excreted via urine as the unchanged parent compound. The biokinetics and metabolism found in the present study confirm previous results (BASF DocID 1987/0429, study submitted with the Annex II Dossier (1995) and evaluated by European authorities and Germany as rapporteur member state). Excretion and metabolism was very similar in both sexes and 63.56% and 64.65% of the dose were excreted as unchanged BAS 351 H in male and female rats, respectively. Hydroxylation of the parent compound resulted in small amounts of metabolite M351H001, which was excreted at levels of 3.57% and 0.58% of the dose in males and females. Metabolite M351H002 was present in male and female rats at even lower levels as its isomer M351H001 (0.10% and 0.05% of the dose). The remaining six metabolites (M351H003, M351H004, M351H005, M351H006, M351H007 and M351H008) were present only in trace amounts.

Report:	II A 5.1.1/3 [redacted]2011(a) 14C-BAS 351 H (Bentazone) - Study on the effect of Probenecid on plasmakinetics in rats BASF DocID 2011/1265806
Date of report:	07-Dec-2011
Testing facility:	[redacted]
Guidelines:	(EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142; OECD 417; EPA 870.7485; JMAFF Guidelines on the Compiling of Test Results on Toxicity - Tests on In Vivo Fate in Animals (2001)
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

This **mechanistic biokinetic study** is focusing on the mechanism of excretion of ^{14}C-labeled BAS 351 H (Bentazone) in female Wistar rats using Probenecid as a competitive inhibitor of renal anion transporters. Two groups of female rats received a single oral target dose of 80 mg/kg bw ^{14}C-BAS 351 H (Bentazone, calculated as Bentazone acid) by gavage either with or without intraperitoneal pretreatment with Probenecid at 150 mg/kg bw. Blood was sampled at various time points up to 72 hours post dosing and the plasma concentrations of radioactivity were determined. The plasma concentrations versus time curves were used to calculate the $AUC_{0-\infty}$ and plasma half-lives.

Since Probenecid is a competitive inhibitor of the OAT, it was of special interest if the pre-treatment with Probenecid leads to a higher internal dose level of BAS 351 H (Bentazone) in blood plasma (AUC) which would be indicative for the active secretion of Bentazone via OAT in the kidney.

Maximum plasma levels were reached after 0.5 hours in the ^{14}C-BAS 351 H (Bentazone) treated group (223.79 µg Eq/g) and after 4 hours in the Probenecid + ^{14}C-BAS 351 H (Bentazone) treated group (125.18 µg Eq/g) and declined thereafter.

The $AUC_{0-\infty}$ in the group treated with ^{14}C- BAS 351 H (Bentazone) alone, was 753 µg Eq x h/g. In the group pretreated with Probenecid, the AUC was calculated to be 1892 µg Eq x h/g.

As can be seen in the table below, thus the AUC in the Probenecid + ^{14}C- BAS 351 H (Bentazone) treated group was increased by a factor of 2.51. This increase was clearly indicative for inhibition of renal excretion of BAS 351 H (Bentazone) by Probenecid.

Target dose of ^{14}C- BAS 351 H (Bentazone) (mg/kg bw)	$AUC_{0-\infty}$ [µg Eq•h/g]		
	^{14}C- BAS 351 H (Bentazone)	Probenecid + ^{14}C- BAS 351 H (Bentazone)	Increase related to ^{14}C- BAS 351 H (Bentazone) group
80	753	1892	2.5 X

The initial and terminal half-lives were nearly doubled from 1.5 and 18.4 hours in the ^{14}C- BAS 351 H (Bentazone) treated group to 2.7 and 25.9 hours in the Probenecid + ^{14}C- BAS 351 H (Bentazone) treated group, respectively. Although being a rather insensitive indicator for excretory saturation phenomena, the data of initial and terminal half-lives in plasma further supported the conclusions drawn from the AUC-data.

In summary, Probenecid pretreatment induced an increase of the AUC and of the initial as well as terminal half lives in ^{14}C-BAS 351 H (Bentazone) treated rats. These results clearly indicate inhibition of renal BAS 351 H (Bentazone) excretion by Probenecid. Since Probenecid pretreatment blocks secretion via the Organic Anion transporter (OAT), it is reasonable to conclude that **bentazone is actively secreted via the saturable Organic Anion transporter**.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

a) Radiolabelled test material:	^{14}C-BAS 351 H (Bentazone) Label: [Phenyl-U]-^{14}C
Description:	not reported
Chemical name (IUPAC):	3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide
Test-substance No.:	08/0637-3
Lot/Batch #:	210-2401
Purity:	Radiochemical purity: > 95 % Specific activity: 5.32 MBq/mg (certificate of Analysis: IL210_2401)
b) Non-radiolabelled test material:	a) BAS 351 H (Bentazone) b) Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na)
Description:	both a and b) solid/ yellowish
Chemical name (IUPAC):	a) 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide b) Sodium 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide
Test-substance No.:	a) 02/0538-3 b) 10/0373-3
Lot/Batch #:	a) COD-001416 b) COD-001417
Purity:	a) 100.0 % ± 1.0 % b) 91.9 % ± 1.0 %, equivalent to 84.2% Bentazone-acid
Stability of test compound:	both a and b: stability under storage conditions was guaranteed until expiry date: 01 Nov 2013; The stability of the test substances in the vehicle was verified by LSC and HPLC-analysis in the experiments by analyses of samples of the test substance preparations before and after its administration.

c) Additional test material: Probenecid

Description:	solid/ white
Chemical name (IUPAC):	not reported
Test-substance No.:	02/0446-3
Lot/Batch #:	BCBC7346V
Purity:	not reported

Stability of test compound: stability under storage conditions was guaranteed until expiry date: 12 Jul 2014
The stability of the test substances in the vehicle was verified by LSC and HPLC-analysis in the experiments by analyses of samples of the test substance preparations before and after its administration.

2. Vehicle and/or positive control: 1 % carboxymethyl cellulose suspension in tap water
For Probenecid: 0.9% saline including 0.5 % Carboxymethyl cellulose containing and 1% Cremophor.

3. Test animals:

Species:	Rat
Strain:	Crl:WI (Han)
Age:	about 11 weeks
Weight at dosing:	Animals of comparable weight experiment 1: females ø 241.7 g experiment 2: females ø 251.6 g (information from report tables)
Source:	Charles River Laboratories, 97633 Sulzfeld, Germany and Charles River laboratories, Inc.
Diet:	Kliba lab diet (mouse / rat “GLP”) pelleted. Origin: Provimi Kliba SA, 4303 Kaiseraugst, Switzerland ad libitum prior to and during the experiments
Water:	tap water ad libitum
Housing:	during acclimatization and prior to the experiment animals were housed in groups in Macrolon Cages (2000P; H Temp (PSU), 2065 cm², Tecniplast); during the experiment individually in steel wire mesh cages
Environmental conditions:	
Temperature:	20 - 24°C;
Humidity:	30 - 70 % relative humidity;
Air change:	not reported
Photoperiod:	6 am to 6 pm;

4. Preparation of dosing solutions

a) Preparation of ^{14}C-BAS 351 H (Bentazone) solution

For the radioactive test substance preparation, ^{14}C-BAS 351 H (Bentazone-acid) was transformed into the Na-salt by addition of equimolar amounts of NaOH. Then, non-radiolabeled test substance Reg.No. 88691 (Bentazone-sodium) was added and mixed with a 1 % carboxymethyl cellulose suspension in tap water. The weight was adjusted to the target dose (80 mg/kg bw, calculated for the acid form of BAS 351 H).
The ratio of radiolabeled to non-radiolabeled test substance is based on the target dose of test substance to the animals and the amount of radioactivity per animal that was about 2 MBq/animal.

Prior to administration, the preparation was sonicated and stirred to produce a homogeneous preparation.

b) Preparation of Probenecid solution

Probenecid (0.5253 g) was dissolved in 35.0073 g 0.9% saline including 0.5 % carboxymethyl cellulose and 1% Cremophore.

The stability, homogeneity and correctness of the concentrations of the test substances in the aqueous vehicle were verified in all experiments by LSC and HPLC-analysis before and after the administrations.

<u>HPLC analysis of the radioactive test substance in the stock solution and the preparation</u>

The analyses of the radioactive test substance in the stock solution and the test substance preparation were performed by HPLC (HP 1100 system; Column: Luna 5µ C18(2) 100A, 250 mm x 3 mm, Eluent: 50% Acetonitril in highly deionized water (acidified with formic acid (1000 + 1mL: v + v)); Flow: 0.8 mL/min; Detection: UV-extinction at 210 nm; HPLC radioactivity monitor LB 509 (cell: YG-150)).

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 05-Jul-2011 (Arrival of animals) –
04-Aug-2011 (last LSC measurement)

2. Animal assignment and treatment:

Two comparative experiments have been performed to elucidate the contribution of the Organic Anion transporter (OAT) in the kidney in the process of excretion of Bentazone (BAS 351 H). The plasmakinetic of radiolabeled ^{14}C-BAS 351 H (Bentazone) in female Wistar rats was investigated with and without Probenecid pretreatment. Probenecid works by competitive inhibition of the OAT in the kidney. It was of special interest if the administration of Probenecid leads to higher internal dose levels of BAS 351 H (Bentazone) in plasma (AUC) after oral dosing which would be indicative for the active secretion of Bentazone via OAT in the kidney.

In experiment 1, the total radioactivity of ^{14}C-BAS 351 H (^{14}C-Bentazone) in plasma (AUC) after a single oral dosing of 80 mg/kg bw via gavage, calculated as Bentazone-acid equivalent, was investigated in 5 female Wistar rats. The administration volume was 10 ml/kg bw.

In experiment 2, 6 female Wistar rats received 150 mg/kg bw Probenecid intraperitoneal 30 min before oral Bentazone administration. The administration volume for the Probenecid pretreatment was 10 ml/kg bw. The following ^{14}C-Bentazone preparation administration was similar to that described in experiment 1.

Sampling
In this set of experiments animals were treated, then placed in steel wire mesh cages and blood samples (100 – 300 µl) were taken from the retro orbital sinus under isoflurane anaesthesia at the following time points or by exanguination (under isoflurane anaesthesia) at the last time point:

0.5;1; 2; 4; 8; 24; 48; 72 hours

Sample preparation for analysis
Aliquots of whole blood samples were inverted several times to ensure homogeneity and then separated into plasma and blood cells by centrifugation. The weights of plasma samples were recorded, and aliquots were mixed by shaking with scintillator (Hionic Fluor cocktail) and analysed by liquid scintillation counting.

Determination of radioactivity in biological material
Total radioactivity was counted in a liquid scintillation counter.

Body Weights
The body weight was determined on the day of administration prior to dosing.

Data processing

Tables presented in the report are computer generated. The group mean and individual data were rounded appropriately for inclusion in the report. As a consequence, calculation of group mean data from the individual data presented in the report will in some instances, yield a minor variation in value.
The calculations of equivalents of test material in the blood plasma were obtained using the following formula (see below).

Key of abbreviations		Dimension
DPM	= disintegrations per minute	[DPM]
LSC	= weight of sample, prepared for liquid scintillation counting (LSC)	[g]
SAM	= weight of organs/Tissue	[g]
AQU	= weight of double distilled water	[g]
ACT	= specific activity of test material	[DPM/µg]
EQUTIS	= equivalents of test material per tissue weight	[µg/g]

$$EQUTIS = \frac{\sum_{n=1}^{n} DPM_n / LSC_n}{n} \; x \; \frac{SAM + AQU}{SAM \; x \; ACT}$$

Analysis of kinetic data:
Analysis of kinetic data was performed based on the group mean values using the PC program system TOPFIT Version 2.0 (1).

II. RESULTS AND DISCUSSION

1. Stability, homogeneity and concentration control analysis of the test substance preparation

The analytical investigations of ^{14}C-BAS 351 H (Bentazone) demonstrated the stability, homogeneity and correctness of the nominal concentrations of the test substance in the preparation during the period of administration. The analytical investigations performed in the context of this study also demonstrated the stability, homogeneity and correctness of the concentrations of Probenecid in the vehicle for the performed experiment.

The test substance preparation was performed with the Na salt of BAS 351 H (Bentazone). An appropriate equivalent factor was applied to calculate the corresponding concentrations for the acid form that was the relevant form for the definition of the target dose levels.

Table 5.1/7 Nominal and analytical concentrations compared to target concentrations

Dose group	Target concentration [mg/g]	Nominal concentration [mg/g]	Analytical results % of nominal concentration (Mean ± SD)
Bentazone	8.0 [1]; 9.5 [2]	9.52 [2]	98.2 ± 0.8 [2]
Probenezid	15.0	15.0	101.7 ± 1.2

[1] 351 H (Bentazone) acid
[2] BAS 351 H (Bentazone) Na salt

2. Plasma levels of 14C- BAS 351 H (bentazone), with and without Probenecid Pre-treatment

All data given are given as ^{14}C- BAS 351 H (Bentazone) acid equivalents. These calculations are based on group mean values.

Following a single oral administration of ^{14}C- BAS 351 H (Bentazone) acid equivalents to female rats at a mean actual dose of 85.09 mg/kg bw, the maximum plasma concentration of 223.79 µg Eq/g occurred 0.5 hours post-dosing (see Table 5.1/8). Afterwards, plasma levels declined to 0.1 µg Eq/g at sacrifice after 72 hours. The initial half-life was calculated to be 1.5 hours (see Table 5.1/9). Terminal half-life was 18.4 hours. The calculated area under the plasma concentration time curve (AUC) was 753 µg Eq*h/g.

In Probenecid pre-treated female rats exposed to a mean single oral actual dose of 84.90 mg/kg bw ^{14}C- BAS 351 H (Bentazone) acid equivalent, the maximum plasma concentration of 125.18 µg Eq/g occurred 4 hours post-dosing. Afterwards, plasma levels declined to 0.1 µg Eq/g at sacrifice after 72 hours. The initial half-life was calculated to be 2.7 hours. Terminal half-life was 25.9 hours. The calculated area under the plasma concentration time curve (AUC) was 1892 µg Eq*h/g.

Table 5.1/8 Mean plasma concentrations of radioactivity after single oral administration of ^{14}C- BAS 351 H (Bentazone) at actual mean dose levels of 85.09 and 84.90 mg/kg bw to female Wistar rats with and without pretreatment with Probenecid

Time [h]	^{14}C-BAS 351 H (Bentazone)	^{14}C-BAS 351 H (Bentazone) + Probenecid pretreatment
0.5	223.79	76.54
1	154.28	95.60
2	104.07	115.85
4	40.81	125.18
8	17.10	118.51
24	0.61	1.03
48	0.17	0.19
72	0.10	0.10

Table 5.1/9 Pharmacokinetic parameters of ^{14}C- BAS 351 H (Bentazone) in female Wistar rats with and without pretreatment with Probenecid

Actual dose	cmax [µg Eq/g]	Tmax [h]	initial half life [h]	terminal half life [h]	$AUC_{0-\infty}$ [µg Eq *h/g]
85 mg/kg bw ^{14}C-BAS 351 H (Bentazone)	223.79	0.5	1.5	18.4	753
85 mg/kg bw ^{14}C-BAS 351 H (Bentazone) + Probenecid pretreatment	125.18	4.0	2.7	25.9	1892
Increase related to ^{14}C- BAS 351 H (Bentazone) group					2.51 X

Comparing the two experiments, an increase of the T^{Max}-value and a decrease in the maximum plasma concentration was observed when ^{14}C-BAS 351 H (Bentazone) dosed female rats were pretreated with Probenecid. This indicates to a certain degree an inhibition of gastrointestinal resorption of ^{14}C-BAS 351 H (Bentazone) by Probenecid.
Otherwise, the AUC $_{0-\infty}$-value of ^{14}C-BAS 351 H (Bentazone), calculated from the plasma concentration versus time curves of the Bentazone-group was 753 µg Eq x h/g, while that of the Probenecid pre-treated group was calculated to be 1892 µg Eq x h/g, thereby showing an increase by a factor of 2.51. This increase of the $AUC_{0-\infty}$-value by Probenecid pre-treatment is clearly indicative for an inhibition of renal excretion of BAS 351 H (Bentazone) by Probenecid.
The before mentioned indications that gastrointestinal uptake seems to be in addition inhibited by Probenecid makes the increase of the AUC after pretreatment with Probenecid even more relevant in respect to inhibition of active renal excretion.

The initial and terminal half-life were nearly doubled from 1.5 and 18.4 hours in the ^{14}C- BAS 351 H (Bentazone) treated group to 2.7 and 25.9 hours in the Probenecid + ^{14}C- BAS 351 H (Bentazone) treated group, respectively. Although being a rather insensitive indicator for excretory saturation phenomena, the data of initial and terminal half-lives in plasma further supported the conclusion that Probenecid pre-treatment inhibits the renal excretion of BAS 351 H.

III. CONCLUSION

Probenecid i.p. pre-treatment of female Wistar rats resulted in an increase of the AUC and of the initial as well as terminal half lives in ^{14}C-BAS 351 H (Bentazone) treated rats. These results clearly indicate inhibition of renal excretion of Bentazone by Probenecid. Since Probenecid is a competitive inhibitor of the Organic Anion transporter (OAT), it is reasonable to conclude that bentazone is actively secreted via the Organic Anion transporter under physiological conditions.

Report:	II A 5.1.1/4 [redacted] 2011(b) 14C-BAS 351 H (Bentazone) - Study on the plasmakinetics in rats BASF DocID 2011/1262233
Date of report:	07-Dec-2011
Testing facility:	[redacted]
Guidelines:	(EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. L 142; OECD 417; EPA 870.7485; JMAFF Guidelines on the Compiling of Test Results on Toxicity - Tests on In Vivo Fate in Animals (2001)
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In this **biokinetic study** the plasma kinetics of ^{14}C-BAS 351 H (Bentazone) in female Wistar rats were examined over the target dose range of 40, 80, 150, 250 and 500 mg/kg bw in order to elucidate a possible onset of saturation of renal excretion, which was already rudimentary discussed in BASF DocID 1987/0429, study submitted with the Annex II Dossier (1995) and evaluated by European authorities and Germany as Rapporteur member state and which in addition is likely to occur as Bentazone was shown in a mechanistic biokinetic study (see chapter 5.1.1/3) to be actively secreted via the saturable organic anion transporter.

For the study, two separate experiments were performed and the dose of 40 mg/kg was performed twice, once in the first and once in the second experiment.
The pharmacokinetic parameters showed a clear dose dependency for the maximum plasma concentrations that were 534.90 and 600.22 µg Eq/g with two T_{Max} values at 0.5 and 4 h for the high dose and 513.14, 353.77 and 185.76 for target dose levels of 250, 150 and 80 mg/kg bw. The low target dose level of 40 mg/kg that was investigated in two experiments revealed maximum plasma concentrations of 109.68 and 85.76 µg Eq/g. For dose levels from 40 to 250 mg/kg bw, the maximum plasma concentrations were reached 0.5 h post dosing.
Initial half lives between 0.69 and 6.15 h demonstrated generally fast excretion of ^{14}C-BAS 351 H (Bentazone) from female rats, dosed with a single oral dose of the test substance.

The calculated AUC values in the experiments were 8731, 3156, 2144 and 502 µg Eq x h /g for actual dose levels of 558.8, 272.7, 165.9 and 84.7 (calculated for the dosed sodium salt of BAS 351 H Bentazone), respectively. For the two dose groups with a target dose of 40 mg/kg bw, AUC values of 303 and 207 were calculated for actual doses of 41.6 and 45.1 mg/kg bw. These variabilities, especially in the low dose group are assessed to reflect the variabilities observed for the different individual rats.

As can be seen from AUC versus dose ratio relationships (AUC ratios of the higher dose levels compared to the AUC of the low dose experiment), the internal dose starts to be overproportional to the oral dose between 84.7 and 165.9 mg/kg bw as shown in the following summary.

Mean Actual dose[1]* [mg/kg bw]	AUC0-∞ [µg Eq *h/g]	Dose ratio to low dose [-]	AUC ratio to AUC of low dose [-]
558.8	8731	13.4	28.8
272.7	3156	6.6	10.4
165.9	2144	**4.0**	**7.1**
84.7	502	**2.0**	**1.7**
41.6 (exp. 1)	303	1.0	1.0

* The respective experiment set 1 is taken to prevent overprediction
[1] Mean Actual dose is related to the sodium salt of BAS 351 H (Bentazone) corresponding to 1.09 x dose (related to the acid form)

This effect may be based on active excretion of the test substance or its metabolites with saturation at higher doses, yielding to overproportional internal doses with increasing dose when a threshold dose (saturation of excretion) is reached. Within the current study, the saturation of excretion starts between actual dose levels of 84.7 and 165.9 mg/kg bw (related to the Bentazone sodium salt).

(BASF DocID 2011/1262233)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

a) Radiolabelled test material: ^{14}C-BAS 351 H (Bentazone)
Label: [Phenyl-U]-^{14}C

Description: not reported
Chemical name (IUPAC): 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide

Test-substance No.: 08/0637-2
Lot/Batch #: 210-2301
Purity: Radiochemical purity: > 96.5 %
Specific activity: 5.15 MBq/mg
(certificate of Analysis: IL210_2301)

b) Non-radiolabelled test material: BAS 351 H (Bentazone)

Description:	solid/ yellowish
Chemical name (IUPAC):	a) 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide
Test-substance No.:	a) 02/0538-2
Lot/Batch #:	a) N 187
Purity:	a) 99% ± 1.0 % (certificate of Analysis: 328663_2)

Stability / Homogeneity: stability under storage conditions was guaranteed until expiry date: 01 Sep 2013;

The stability of the test substances in the vehicle was verified by analysis in the experiments at least in samples that were taken before and after test substance administration.
The concentrations, homogeneities and radiochemical purities of the test substance preparations were verified by liquid scintillation counting and HPLC analyses in the experiments.

2. Vehicle and/or positive control: 0.5% carboxymethyl cellulose in tap water

3. Test animals:

Species:	Rat
Strain:	Crl:WI (Han) (SPF)
Age:	about 12-14 weeks
Weight at dosing:	females bw range 200-283.0g (information from report tables)
Health status:	The health status of the animals was checked prior to and during the experiment at least once daily.
Source:	Charles River Laboratories, 97633 Sulzfeld, Germany and Charles River laboratories, Inc.
Diet:	Kliba lab diet (mouse / rat “GLP”) pelleted. Origin: Provimi Kliba SA, 4303 Kaiseraugst, Switzerland ad libitum prior to and during the experiments
Water:	tap water ad libitum
Housing:	during acclimatization and prior to the experiment animals were housed in groups in Macrolon Cages (2000P; H Temp (PSU), 2065 cm², Tecniplast); during the experiment individually in steel wire mesh cages
Environmental conditions:	
Temperature:	20 - 24°C;
Humidity:	30 - 70 % relative humidity;
Air change:	not reported
Photoperiod:	6 am to 6 pm;

4. Preparation of ^{14}C-BAS 351 H (Bentazone) solution

For the radioactive test substance preparation, an appropriate aliquot of ^{14}C-BAS 351 H (Bentazone-acid) in acetonitrile was evaporated to dryness and respective amounts of non-radiolabeled test substance and 0.5 % carboxymethyl cellulose in tap water were added. The ratio of radiolabeled to non-radiolabeled test substance is based on the target dose of test substance to the animals and the amount of radioactivity per animal that was about 2 MBq/animal.

The preparation was stirred in order to produce homogeneous preparation. At least before start and at the end of the administration, samples were taken to determine the amount of radioactivity in the preparation and to demonstrate the correct concentration of the test substance and its homogeneity and its radiochemical purity via LSC and HPLC-analyses. The stability, homogeneity and correctness of the concentrations of the test substance in the aqueous vehicle were be verified in all experiments by LSC and HPLC-analyses.

HPLC analysis of the radioactive test substance in the stock solution and the preparation
The analyses of the radioactive test substance in the stock solution and the test substance preparation were performed by HPLC (HP 1100 system; Column: Luna 5µ C18(2) 100A, 250 mm x 3 mm, Eluent: 50% Acetonitril in highly deionized water (acidified with formic acid (1000 + 1mL: v + v)); Flow: 0.8 mL/min; Detection: UV-extinction at 210 nm; HPLC radioactivity monitor LB 509 (cell: YG-150)).

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 13-Jul-2010 (1st arrival of animals) –
06-Jan-2011 (last LSC measurement)
22-Jul-2010 - 17-Dec-2010 (Animal experimental phase)

2. Animal assignment and treatment:

In order to study the biokinetics of ^{14}C-BAS 351 H (Bentazone) in female Wistar rats and thereby elucidate the potential onset of saturation of renal excretion, two supplementing experiments have been performed.

In experiment 1, the total radioactivity of ^{14}C-BAS 351 H (^{14}C-Bentazone) in blood and plasma (AUC) after a single oral dosing of 40, 150 and 500 mg/kg bw via gavage (calculated as acid form) was investigated in each 4 female Wistar rats.
In experiment 2, further dosing under the same conditions as in experiment 1 of 40, 80 and 250 mg/kg bw (calculated as acid form) was investigated in 8, 4 and 4 female Wistar rats, respectively.

It should be taken into account that based on the ratio of the molecular weights of the sodium salt and the acid-form of the test substance, the target dose levels are higher for the sodium salt (see Table 5.1/10).

Table 5.1/10 Dosing groups within the biokinetics study with ^{14}C-BAS 351 H

Test group	Dose of labeled material - (mg/kg bw)		Number of female rats	Remarks
	Acid form	Sodium salt		
Blood/plasma level experiments				
Experiment 1	40, 150, 500	43.6, 163.7, 545.5	each 4	Retro orbital blood sampling at 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 120, 144 hours. Exsanguinations at 168 hours.
Experiment 2	40, 80, 250	43.6, 87.3 272.8	8/4/4	

The administration volume in each experiment was 10 ml/kg bw.

Sampling
In this set of experiments animals were treated, then placed in steel wire mesh cages and blood samples (100 – 300 µl) were taken from the retro orbital sinus under isoflurane anaesthesia at the following time points or by exanguination (under isoflurane anaesthesia) at the last time point:

0.5; 1; 2; 4; 8; 24; 48; 72; 96; 120; 144; 168 hours

Sample preparation for analysis
Aliquots of whole blood samples were inverted several times to ensure homogeneity and then separated into plasma and blood cells by centrifugation.

The weights of plasma samples were recorded, and aliquots were mixed by shaking with scintillator (Hionic Fluor cocktail) and analysed by liquid scintillation counting.

The total weights for blood cells were recorded; samples were solubilised with Soluene®-350 (Perkin Elmer) at 30 °C over night followed by the addition of 1 mL of iso-propanol and bleaching by addition of about 1 mL Hydroperoxide (30% H_2O_2). The samples were incubated over night and afterwards mixed by shaking with scintillator (Hionic Fluor cocktail) and analysed by liquid scintillation counting.

Determination of radioactivity in biological material
Total radioactivity was counted in a liquid scintillation counter.

Body Weights
The body weight was determined on the day of administration prior to dosing.

Data processing

Tables presented in the report are computer generated. The group mean and individual data were rounded appropriately for inclusion in the report. As a consequence, calculation of group mean data from the individual data presented in the report will in some instances, yield a minor variation in value.

Calculations were performed using formula I (see below):

Key of abbreviations		Dimension
DPM	= disintegrations per minute	[DPM]
LSC	= weight of sample, prepared for liquid scintillation counting (LSC)	[g]
SOL	= weight of soluene	[g]
SAM	= weight of organs/Tissue	[g]
AQU	= weight of double distilled water	[g]
ACT	= specific activity of test material	[DPM/µg]
EQUTIS	= equivalents of test material per tissue weight	[µg/g]
D_{rad}	= dose of radioactivity administered	[DPM]

Formula I

$$EQUTIS = \frac{\sum_{n=1}^{n} DPM_n / LSC_n}{n} \; x \; \frac{SAM + AQU}{SAM \; x \; ACT}$$

Analysis of kinetic data:
Analysis of kinetic data was performed based on the group mean values using the PC program system TOPFIT Version 2.0 (1).

II. RESULTS AND DISCUSSION

1. Stability, homogeneity and concentration control analysis of the test substance preparation

The analytical investigations of ^{14}C- BAS 351 H (Bentazone) demonstrated the stability, homogeneity and correctness of the nominal concentrations of the test substance in the preparation for all performed experiments.
The nominal and analytical concentrations compared to target concentrations are given below.

Dose group	Target concentration [mg/g]	Nominal concentration [mg/g]	Analytical results % of nominal concentration (Mean ± SD)
40 mg/kg bw	4.0	3.98	96.2 ± 0.6
40 mg/kg bw (2nd experiment)	4.0	4.10	102.8 ± 1.3
80 mg/kg bw	8.0	8.04	98.7 ± 4.0
150 mg/kg bw	15.0	14.9	91.8 ± 1.9
250 mg/kg bw	25.0	25.0	95.9 ± 4.9
500 mg/kg bw	50.0	49.34	95.4 ± 0.4

The mean concentrations found were in the range of 91.8 to 102.8 % of the nominal concentrations. The standard deviation as range of homogeneity varied in the range of 0.4 to 4.9 %. At least one sample per time point (before, during and after administration) was taken to calculate the analytical results.

2. Plasma and blood levels of 14C- BAS 351 H (bentazone)

The single oral administration of ^{14}C-BAS 351 H to female Wistar rats at target dose levels of 500, 250, 150, 80 and 40 mg/kg bw (two experiments) calculated as Bentazone-acid equivalents resulted in actual mean doses (calculated for the dosed sodium salt of BAS 351 H) of 558.8, 272.7, 165.9, 84.7, 41.6 and 45.1 mg/kg bw.
In female rats exposed to a target dose of 500 mg/kg bw ^{14}C- BAS 351 H (Bentazone), the maximum mean plasma concentrations of 534.90 µg Eq/g and 600.22 µg Eq/g occurred 0.5 and 4 hours post-dosing. The lower target doses of 250, 150, 80 and 40 (experiment 1 & 2) mg/kg bw ^{14}C- BAS 351 H resulted in maximum mean plasma concentrations of 513.14 µg Eq/g, 353.77 µg Eq/g, 185.76 and 109.68 & 85.76 µg Eq/g, respectively. Afterwards plasma levels declined to 0.4 µg Eq/g in females of the high dose group, as well as to 0.16, 0.17, 0.05 and 0.06 µg and 0.02 Eq/g in the 250, 150, 80 and 40 (exp.1 & 2) mg/kg bw groups, respectively (see Table 5.1/11).

Table 5.1/11 Mean plasma concentration of radioactivity after single oral administration of ^{14}C BAS 351 H at target dose levels of 500, 250, 150, 80 and 40 mg/kg bw to female Wistar rats

	Results expressed in µg Eq/g plasma					
Target dose[1]	**500 mg/kg bw**	**250 mg/kg bw**	**150 mg/kg bw**	**80 mg/kg bw**	**40 mg/kg bw** (exp. 1)*	**40 mg/kg bw** (exp. 2)*
Mean Actual dose[2]	558.8 mg/kg bw	272.7 mg/kg bw	165.9 mg/kg bw	84.7 mg/kg bw	41.6 mg/kg bw	45.1 mg/kg bw
Time [h]						
0.5	534.90	513.14	353.77	185.76	109.86	85.76
1	506.62	429.04	309.04	130.89	61.20	39.30
2	495.61	332.48	276.27	50.97	44.24	18.14
4	600.22	214.09	130.37	21.41	24.33	6.17
8	398.92	121.46	72.43	9.81	3.17	5.37
24	63.79	2.55	7.01	0.43	0.50	0.28
48	1.23	0.53	0.48	0.18	0.15	0.11
72	0.67	0.46	0.24	0.22	0.09	0.08
96	0.72	0.28	0.25	0.11	0.08	0.05
120	0.51	0.23	0.22	0.06	0.08	0.04
144	0.52	0.17	0.17	0.04	0.07	0.04
168	0.40	0.16	0.17	0.05	0.06	0.02

* The respective experiment set 1 and 2 is indicated in brackets

[1] Target dose is related to the acid form of BAS 351 H (Bentazone)

[2] Mean Actual dose is related to the sodium salt of BAS 351 H (Bentazone) corresponding to 1.09 x dose (related to the acid form)

Calculated initial half lives were 6.15, 2.84, 2.45, 0.79, 1.23 and 0.69 hours in the 500, 250, 150, 80 and the two sets of 40 mg/kg bw dose groups, respectively. Terminal half-lives ranged between 60 and 109.13 hours for all dose groups. These calculations are based on group mean values. The calculated area under the plasma concentration time curves (AUC) are shown in Table 5.1/12.

Table 5.1/12 Pharmacokinetic parameters of ^{14}C-BAS 351 H in female Wistar rats

Target Dose[1]* [mg/kg bw]	Actual Dose[2] [mg/kg bw]	c_{max} [µg Eq/g]	T_{max} [hour]	initial half life [hour]	terminal half life [hour]	AUC [µg Eq x h/g]
500(exp. 1)	558.8	534.90; 600.22	0.5; 4	6.15	86.62	8731
250 (exp. 2)	272.7	513.14	0.5	2.84	83.97	3156
150(exp. 1)	165.9	353.77	0.5	2.45	91.66	2144
80 (exp. 2)	84.7	185.76	0.5	0.79	60.04	502
40 (exp. 1)	41.6	109.68	0.5	1.23	109.13	303
40 (exp. 2)	45.1	85.76	0.5	0.69	60.52	207

* The respective experiment set 1 and 2 is indicated in brackets

[1] Target dose is related to the acid form of BAS 351 H (Bentazone)

[2] Mean Actual dose is related to the sodium salt of BAS 351 H (Bentazone) corresponding to 1.09 x dose (related to the acid form)

The different dose groups showed nearly constant blood/plasma ratio values < 1 up to 24 h post-dosing. Thereafter, the blood/plasma ratio increased steadily. A blood/plasma ratio of around 1 was reached in all dose groups between 48 and 72 h. Maximum mean blood/plasma ratios ranged from 1.83 and 2.75 and occurred 144 or 168 h post dosing.

As can be seen from AUC versus dose ratio relationships (AUC ratios of the higher dose levels compared to the AUC of the low dose experiments), the internal dose is overproportional to the oral dose (see Table 5.1/13). This was already seen in the former plasmakinetic study in rats performed with only a low and a high dose (see DAR, chapter 8.5.1.1.1, Doc ID 1987/0429) and confirms the non-linear region in disposition at higher doses. The normally observed tendency of lower AUC/unit dose levels at higher external doses due to saturation of absorption is not seen with Bentazone, but the opposite of higher AUC/unit dose, indicating a saturation of excretion.

Table 5.1/13 AUC/dose ratios after single oral administration of ^{14}C-BAS 351 H (Bentazone) at target dose levels of 500; 250; 150, 80 and 40 mg/kg bw to female rats

Mean Actual dose[1]* [mg/kg bw]	AUC [µgEq x h/g]	AUC/dose [µgEq x h x kg bw/g x mg]
558.8	8731	15.6
272.7	3156	11.6
165.9	2144	12.9
84.7	502	5.9
45.1(exp. 2)	207	4.6
41.6 (exp. 1)	303	7.3

* The respective experiment set 1 and 2 is indicated in brackets

[1] Mean Actual dose is related to the sodium salt of BAS 351 H (Bentazone) corresponding to 1.09 x dose (related to the acid form)

In order to avoid overprediction of saturation, the comparison of AUC versus dose ratio relationships is performed versus the experiment with the higher AUC at the low dose level (original low dose experiment 1). Increasing the dose by a factor of 2 (from 41.6 to 84.7 mg/kg bw) results in an increase of the AUC-values by a factor of 1.7. Increasing the dose by a factor of 13.4 (from 41.6 to 558.8 mg/kg bw) results in an overproportional increase of the AUC-values by a factor of 28.8 (see Table 5.1/14). This effect may be based on active excretion of the test substance or its metabolites with saturation at higher doses, yielding to overproportional internal doses with increasing dose when a threshold dose (saturation of excretion) is reached. Within the current study, the saturation of excretion starts between actual dose levels of 84.7 and 165.9 mg'/kg bw.

Table 5.1/14 Dose versus AUC ratios after single oral administration of ^{14}C- BAS 351 H (Bentazone) to female rats at target dose levels of 500; 250; 150 and 80 mg/kg bw compared to a target dose level of 40 mg/kg bw (Experiment 1)

Mean Actual dose[1]* [mg/kg bw]	AUC0-∞ [µg Eq *h/g]	Dose ratio to low dose [-]	AUC ratio to AUC of low dose [-]
558.8	8731	13.4	28.8
272.7	3156	6.6	10.4
165.9	2144	4.0	7.1
84.7	502	2.0	1.7
41.6 (exp. 1)	303	1.0	1.0

* The respective experiment set 1 is indicated in brackets and taken to prevent overprediction
[1] Mean Actual dose is related to the sodium salt of BAS 351 H (Bentazone) corresponding to 1.09 x dose (related to the acid form)

III. CONCLUSION

Taken together, the AUC versus dose ratio relationships indicate that the internal dose is overproportional to the oral dose. This effect may be based on active excretion of the test substance or its metabolites with saturation at higher doses, yielding to overproportional internal doses with increasing dose when a threshold dose (saturation of excretion) is reached. Within the current study, **saturation of excretion in rats starts between actual dose levels of 84.7 and 165.9 mg/kg bw** (calculated as bentazone-sodium corresponding to 1.09 x dose of Bentazone acid).

5.1.2 Toxicokinetic studies - Second single dose, oral route, in rats

No new studies were performed. All information available has been presented in the original Annex II Dossier (1995) and is evaluated by European authorities and Germany as Rapporteur member state.

5.1.3 Toxicokinetic studies - Repeated dose, oral route, in rats

No new studies were performed. All information available has been presented in the original Annex II Dossier (1995) and is evaluated by European authorities and Germany as Rapporteur member state.

5.2 Acute toxicity

Studies presented in the original Annex II Dossier (1995): Bentazone has been tested in various species and via different routes of administration, mainly as free acid, but additionally as sodium salt. All studies are scientifically valid; however, partially the studies have been conducted before the release of study guidelines and are without GLP according to the usual practice in those days. Although no individual data are reported, the reports are sufficiently detailed for key data. Deviations in the studies are considered to be minimal and a repeat of studies according to current standard would not provide any additional insight. These studies have been evaluated by European authorities and Germany as Rapporteur member state in 1998 (European Commission Peer Review Program, BASF Doc ID 1998/1001178) and are, for the convenience of the reviewer, listed in Table 5.2/1.

Acute studies with **Bentazone as free acid** revealed a low to moderate acute oral toxicity in rats, mice, guinea pigs, cats and dogs. The acute oral toxicity in rats ranged between 850 and 2000 mg/kg bw and, according to latest studies, the LD50 in female rats as most sensitive gender was 1470 mg/kg bw. The EU agreed LD_{50} (oral, rat) for bentazone was set to 1400 - 1800 mg/kg bw (EC Review report, 2000). Bentazone-acid is virtually nontoxic via the dermal route (LD_{50} > 5.000 mg/kg bw) and by inhalation (LC_{50} (4 hour) > 5.1 mg/l). The irritating effects were transient and very slight on the skin. Irritating effects on the eyes were also reversible but moderate, resulting in the classification of Bentazone-acid for its eye irritating property. Furthermore Bentazone-acid exhibited sensitizing properties in the Magnusson-Kligmann-Test. The tests on **Bentazone-sodium** revealed similar acute oral toxicity to rats and comparable sensitizing properties in an Open Epicutaneous Test on guinea pigs, with dilutions of bentazone-sodium of 10 % and below showing no sensitization in guinea pig.

Table 5.2/1 Summary of peer-reviewed acute toxicity studies including irritation, sensitization and other acute studies with BAS 351 H (core studies are marked bold)

Reference	Species (Strain)	Sex	Test substance	Endpoint
LD_{50} – oral route				
DocID 1969/0013	Rat (Sprague-Dawley)	male and female	bentazone	LD_{50} about 850 mg/kg bw
DocID 1972/051	Rat (Sprague-Dawley)	male and female	bentazone	LD_{50} = 1,050 mg/kg bw
DocID 1973/022	Rat (Sprague-Dawley)	male and female	bentazone	LD_{50}= 1,220 mg/kg bw
DocID 1973/023	**Rat (Sprague-Dawley)**	**male and female**	**bentazone-sodium salt**	**LD_{50}= 1,480 mg/kg bw equivalent to LD_{50}= 1,356 mg/kg bw (free acid)**
DocID 1978/053	Rat (CRJ:SD)	male and female	bentazone	LD_{50}=2,340 mg/kg bw (♂) LD_{50}=2,470 mg/kg bw (♁)
DocID 1983/113	Rat (Wistar)	male and female	bentazone	LD_{50}= 1,710 mg/kg bw LD_{50}= 1,780 mg/kg bw (♂) LD_{50}= 1,790 mg/kg bw (♁)
DocID 1983/114	**Rat (Wistar)**	**male and female**	**bentazone**	**LD_{50}= 1,640 mg/kg bw** **LD_{50}= 1,780 mg/kg bw (♂)** **LD_{50}=1,470 mg/kg bw (♁)**
DocID 1974/035	Guinea pigs (Pirbright White)	male and female	bentazone-sodium salt	LD_{50}= 1,100 mg/kg bw Equivalent to LD_{50}= 1,000 mg/kg bw (free acid)
DocID 1991/10147	Guinea pigs (Pirbright White)	male and female	bentazone	LD_{50} about 1,100 mg/kg bw
DocID 1970/016	Cats	male and female	bentazone	LD_{50} about 500 mg/kg bw
DocID 1970/017	Dog (Beagle)	male and female	bentazone	LD_{50} derivation was not possible
DocID 1969/005	Rabbits	male and female	bentazone	No LD_{50} calculation performed
DocID 1993/11411	Rabbit (New Zealand whites)	male and female	bentazone	LD_{50}= 1,139 mg/kg bw
LD_{50} – dermal route				
DocID 1969/002	Rat (Sprague-Dawley)	male and female	bentazone	LD_{50}>2,500 mg/kg bw
DocID 1978/055	**Rat (CRJ:SD)**	**male and female**	**bentazone**	**LD_{50}>5,000 mg/kg bw**
LC_{50} –inhalation				
DocID 1969/003	Rat (Sprague-Dawley)	male and female	Bentazone (8-hour)	No mortalities
DocID 1986/220	**Rat (Wistar)**	**male and female**	**bentazone (4-hour, dust)**	**LC_{50}> 5.1 mg/L**
Skin Irritation				

Table 5.2/1 Summary of peer-reviewed acute toxicity studies including irritation, sensitization and other acute studies with BAS 351 H (core studies are marked bold)

Reference	Species (Strain)	Sex	Test substance	Endpoint
DocID 1969/003	**Rabbit (White Vienna)**	**male and female**	**bentazone**	**No skin irritant**
Eye Irritation				
DocID 1983/083	**Rabbit (White Vienna)**	**male and female**	**bentazone**	**Irritating to eyes**
Skin sensitization				
DocID 1986/195 (Maximisation test)	**Guinea pig (Pirbright White)**	**females**	**bentazone**	**Sensitizing**
DocID 1986/221 DocID 1986/347 DocID 1977/10334 (OET)	Guinea pig (Pirbright White)		bentazone-sodium formulation 600 g/L	Sensitizing at concentrations of 50 % aqueous dilution; Not sensitizing up to 10% aqueous dilution
Other application routes - Subcutaneous				
DocID 1978/053	Rat (Sprague-Dawley, CRJ)	male and female	bentazone	LD_{50}= 970 mg/kg bw (♂) LD_{50}= 975 mg/kg bw (♁)
DocID 1978/054	Mice CRJ:ICR	male and female	bentazone	LD_{50}= 655 mg/kg bw (♂) LD_{50}= 580 mg/kg bw (♁)
Other application routes - Intraperitoneal				
DocID 1972/10130	Rat Sprague-Dawley	male and female	bentazone	LD_{50} = 344 mg/kg bw
DocID 1978/053	Rat (Sprague-Dawley, CRJ)	male and female	bentazone	LD_{50}= 403 mg/kg bw (♂) LD_{50}= 407 mg/kg bw (♁)
DocID 1983/161	Rat Wistar	male and female	bentazone	316 mg/kg bw < LD_{50} < 383 mg/kg bw
DocID 1969/018	Mice, NMRI	male and female	bentazone	LD_{50} about 400 mg/kg bw
DocID 1978/054	Mice CRJ:ICR	male and female	bentazone	LD_{50}= 494 mg/kg bw (♂) LD_{50}= 505 mg/kg bw (♁)

Submission of not yet peer-reviewed studies in this AIRII-Dossier:
No new acute in vivo studies were performed with Bentazone for submission within the AIR II process. In accordance with the requirements of Commission Regulation SANCO/11802/2010 an in vitro NRU-Phototoxicity study in Balb/c 3T3 cells has been performed and is given in detail under chapter 5.2.7. According to this study Bentazone is not phototoxic.

Table 5.2/2 Phototoxicity study with BAS 351 H for evaluation in the AIR 2 process

Study type	Test system	Substance Dose / concentr. range (batch / purity)	Result	Reference (BASF DocID)
NRU-Phototoxicity study	Balb/c 3T3 cells clone A31	Bentazone 4.6-10.0-21.5-46.4-100.0-215.4-464.2-1000 µg/mL (COD-001416 / 100%)	Photo-Irritancy-Factor = *1 > Not phototoxic	AIR2-Dossier II A 5.2.7/1 Cetto V.& Landsiedel R., 2011 (a); DocID 2011/1110261 and 2011/1284096 (Amend.)

In summery, no changes in classification for acute toxic effects are considered necessary for Bentazone, BAS 351 H. Bentazone has to be classified according to GHS-classification criteria laid down in the Council Regulation 1272/2008/EC and OECD Globally Harmonized System (GHS, 2009) with the following hazard code / statement:

- Acute Tox.4 (orl) (H 302) Harmful if swallowed
- Eye Irrit. 2 (H 319) Causes serious eye irritation
- Skin Sens. 1 (H 317) May cause an allergic skin reaction

The endpoints which were fixed in the European Commission Review report for the active substance bentazone (7585/VI/97-final from 30 November 2000) for acute toxicity are still valid and might be supplemented with regard to the Phototoxicity study.

EU agreed Endpoint for bentazone (EC Review report, 2000)

Rat LD_{50} oral:	1400 - 1800 mg/kg bw
Rat LD_{50} dermal:	> 5000 mg/kg bw
Rat LC_{50} inhalation:	> 5.1 mg/L
Skin irritation:	non irritant
Eye irritation:	irritant
Skin sensitization (test method used and result):	Sensitizer (M&K)

5.2.1 Acute oral toxicity

No new studies were performed. All information available is presented in the original Annex II Dossier (1995), has been evaluated by European authorities and Germany as Rapporteur member state and the endpoint was fixed in the European Commission Review report for the active substance bentazone (7585/VI/97-final from 30 November 2000).

EU agreed Endpoint for bentazone (EC Review report, 2000)

Rat LD_{50} oral:	1400 - 1800 mg/kg bw

Report:	II A 5.2.1/1 Cetto V., Landsiedel R. 2011(b) BAS 351 H (Bentazone) - In vitro 3T3 NRU phototoxicity test BASF DocID 2011/1110261
Date of report:	05-Jul-2011
Testing facility:	BASF SE; Ludwigshafen/Rhein; Germany Fed.Rep.
Guidelines:	OECD 432 (2004) In vitro 3T3 NRU Phototoxicity test; (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.41 No. L 142
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	II A 5.2.1/2 Cetto V., Landsiedel R. 2012(b) Amendment No.1 - BAS 351 H (Bentazone) - In vitro 3T3 NRU phototoxicity test BASF DocID 2011/1284096
Date of report:	25-Jan-2012
Testing facility:	BASF SE; Ludwigshafen/Rhein; Germany Fed.Rep.
Guidelines:	OECD 432 (2004) In vitro 3T3 NRU Phototoxicity test; (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.41 No. L 142
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Note: The original report refers to a study in which the stability of the test compound in H2O and DMSO was tested in a comparable batch. These analyses were not performed under GLP-conditions. The Amendment refers to an older study (Chromosome Aberration Doc ID 1987/0169) which is evaluated in the Monograph as acceptable and in which the stability in DMSO is documented. The stability in water is given for Bentazone (see Review Report for the active substance bentazone, Endpoint Hydrolytic Stability). Furthermore the reanalysis to confirm the stability of the substance is provided.

5.2.2 Acute percutaneous toxicity

No new studies were performed. All information available is presented in the original Annex II Dossier (1995), has been evaluated by European authorities and Germany as Rapporteur member state and the endpoint was fixed in the European Commission Review report for the active substance bentazone (7585/VI/97-final from 30 November 2000).

EU agreed Endpoint for bentazone (EC Review report, 2000)

Rat LD_{50} dermal:	> 5000 mg/kg bw

5.2.3 Acute inhalation toxicity

No new studies were performed. All information available is presented in the original Annex II Dossier (1995), has been evaluated by European authorities and Germany as Rapporteur member state and the endpoint was fixed in the European Commission Review report for the active substance bentazone (7585/VI/97-final from 30 November 2000).

EU agreed Endpoint for bentazone (EC Review report, 2000)

Rat LC_{50} inhalation:	> 5.1 mg/L

5.2.4 Skin irritation

No new studies were performed. All information available is presented in the original Annex II Dossier (1995), has been evaluated by European authorities and Germany as Rapporteur member state and the endpoint was fixed in the European Commission Review report for the active substance bentazone (7585/VI/97-final from 30 November 2000).

EU agreed Endpoint for bentazone (EC Review report, 2000)

Skin irritation:	non irritant

5.2.5 Eye Irritation

No new studies were performed. All information available is presented in the original Annex II Dossier (1995), has been evaluated by European authorities and Germany as Rapporteur member state and the endpoint was fixed in the European Commission Review report for the active substance bentazone (7585/VI/97-final from 30 November 2000).

EU agreed Endpoint for bentazone (EC Review report, 2000)

Eye irritation:	irritant

5.2.6 Skin sensitization

No new studies were performed. All information available is presented in the original Annex II Dossier (1995), has been evaluated by European authorities and Germany as Rapporteur member state and the endpoint was fixed in the European Commission Review report for the active substance bentazone (7585/VI/97-final from 30 November 2000).

EU agreed Endpoint for bentazone (EC Review report, 2000)

Skin sensitization (test method used and result):	Sensitizer (M&K)

5.2.7 Potentiation/interactions of multiple active ingredients or products

Not applicable.

Phototoxicity

Executive Summary

BAS 351 H (Bentazone as free acid) (Batch COD-001416; purity 100%) was tested for its ability to induce phototoxic effects in Balb/c 3T3 cells in vitro. The photo-cytotoxicity was estimated by the means of the Neutral Red Uptake (NRU) method. Three independent experiments were carried out, all with and without irradiation with an UVA source, of which the 2nd Experiment was judged invalid due to lacking the predefined criteria of acceptance (data not shown). Vehicle and positive controls were included in each experiment.
BAS 351 H was tested up to the limit concentration with and without UVA irradiation at concentrations of 4.6, 10.0, 21.5, 46.4, 100.0, 215.4 and 1000.0 µg/mL.
In this study, cytotoxicity was observed only in the 1st Experiment from 464.2 µg/mL onward without UVA irradiation. In addition no precipitation of the test substance in the vehicle DMSO was observed both with and without UVA irradiation. On the basis from the results of the present study, the test substance was predicted to have no phototoxic potential (PIF = *1) indicated by Neutral Red Uptake method.
The positive control Chlorpromazine led to the expected cytotoxicity both with and without UVA irradiation (PIF: 63.2 and 74.9), thus demonstrating the sensitivity of the test system.

Thus, under the experimental conditions of this study, BAS 351 H (Bentazone) is considered **not to be a phototoxic substance** in the in vitro 3T3 NRU Phototoxicity Test using Balb/c 3T3 cells.

(BASF DocID 2011/1110261)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material BAS 351 H (Bentazone)

Description: Solid, yellowish

Lot/Batch #: COD-001416

Purity: 100%

Stability of test compound: The test substance was guaranteed to be stable until 01. Nov 2013, thus over the study period under storage conditions. The homogeneity of the test substance was ensured by the high purity and by mixing prior to preparation of test substance solutions.
The test substance is soluble in DMSO and stable in DMSO as tested in previous studies (see Doc ID 1987/0169) and stable in water at room temperature (see List of Endpoints in the Review Report for the active substance bentazone, Endpoint Hydrolytic Stability).

Solvent used: Dimethylsulfoxide (DMSO)

2. Control Materials:

Vehicle control: The vehicle control cultures with and without irradiation only contained the vehicle used for the test substance at the same concentration and volume as used for the test substance and the positive control.

Solvent/final concentration: 1% (v/v) in PBS

Positive control compounds: Chlorpromazine (CPU) was dissolved in DMSO

A complete 96-well plate containing 8 concentrations was performed in parallel to demonstrate sensitivity of the test method.

Without irradiation	1.9-3.8-7.5-15-30-60-90-180 µg/mL
With irradiation	0.03-0.05-0.1-0.2-0.4-0.8-1.6-3.2 µg/mL

3. Test organisms: The Balb/c 3T3, clone A31, cell line was isolated from the muscle tissue of mouse embryo. This fibroblast cell line has a high proliferation and a high plating efficiency of untreated cells both necessary for the appropriate performance of the study. The Balb/c 3T3 cell line which was used in this experiment was obtained from the “European Collection of Cell Cultures” Salisbury, Wiltshire SP4 OJG, UK (date 09 Aug 2006) and is stored at -196°C (liquid nitrogen).

4. Culture media and reagents:

Culture medium:	Dulbecco's Modified Eagle's Medium (DMEM) supplemented with - 10% (v/v) newborn calf serum (NCBS) - 4 mM L-glutamine - 100 IU penicillin - 100 µg/mL streptomycin
Neutral Red solution:	- 0.4 g Neutral Red powder (NR; Sigma N4638) - 100 mL deionized water
Neutral Red medium:	- 1 mL Neutral Red solution - 79 mL culture medium (DMEM incl. supplements) Incubated overnight at 37° C with 5% CO_2 and filtered with a 0.22 µm filter prior to use.
Other solutions and reagents:	- phosphate buffered saline (PBS) without Ca/Mg - trypsin/EDTA solution (0.05%; 0.02%) - Neutral Red desorb solution (1 mL acetic acid, 50 mL ethanol, 49 mL deionized water)

5. Irradiation source: The Sol 500 solar simulator (Dr. Hönle AG, 82166 Gräfelfing, Germany) used with filter H1 produced wavelength > 320 nm. The exposure rates were determined with UV-meter RM-21 (Dr. Gröbel GmbH, 76275 Ettlingen, Germany).

6. Test concentrations:

Pretest:	No pretest for dose selection was performed in this study.
NRU test conditions:	An appropriate amount of test article substance was taken up in the vehicle and shaken thoroughly to get a clear solution and diluted in accordance with the planned doses under light protection conditions immediately before administration. The experiment was performed in 96 well plates in 3 independent experiments (2nd experiment was judged invalid) each with 6 replicates per concentration with and without irradiation. The test substance concentrations were: 4.6-10.0-21.5-46.4-100.0-215.4-464.2-1000 µg/mL

B. TEST PERFORMANCE:

1. Dates of experimental work: 29-Mar-2011 - 16-May-2011

2. NRU Phototoxicity test:

Two 96 well-plates per substance (test substance or positive control) were used for cultivation of cells (1.0 x 10^4 cells/well in the 1st experiment, and 1.5 x 10^4 cells/well in the 3rd experiment). After an attachment period of about 24 hours the cells were treated with the respective substance (8 concentrations each with 6 replicates as indicated above) and the vehicle control in parallel for 1 hour in the dark (5% (v/v) CO_2, 37° C). Then, one microtiterplate per substance was irradiated for 50 minutes with UVA (UV intensity underneath the lid 1.5 - 2.1 mW/cm^2 = 5 J/cm²) whereas the respective reference plate was kept in the dark for the same period. After test substance removal the cells were incubated in culture medium overnight and thereafter in Neutral Red medium for additional 3 hours, each step under light protected conditions in the lab to prevent uncontrolled photo activation. Thereafter, the cells were washed, the dye was extracted by Neutral Red desorb solution. Cytotoxicity was determined by measuring the Neutral Red Uptake using a microplate reader (Perkin Elmer, Waltham, Massachusetts, US; Wallac 1420 multilabel counter) equipped with a 550 nm filter to read the absorption of the extracted dye. The absorption shows a linear relationship with the number of surviving cells.
Additionally the following parameters were measured: pH, osmolarity, solubility and cell morphology.

3. Evaluation/Assessment

For the assessment of the phototoxic potential of a compound two prediction models are currently available:

- The Photo-Irritancy-Factor Prediction model for substances which allow the comparison of two equi-effective concentrations (EC_{50}) in the concurrently performed experiments in the presence and absence of light. This model includes the special case of absence of cytotoxicity in the presence and absence of light for substances obviously showing no phototoxic potential.
- The Mean Photo Effect prediction model which is used if no equi-effective concentrations (EC_{50}) are obtained in the absence and presence of UV light.

3.1 Cytotoxicity

The mean absorbance values obtained for each test group of every plate were used to calculate the percentage of cell viability relative to the respective vehicle control, which is arbitrariliy set at 100 %.

$$Cytotoxicity\,[\%] = \frac{\text{Absorbance}_{\text{mean}}\ \text{of the test group}}{\text{Absorbance}_{\text{mean}}\ \text{of the vehicle control}} \times 100$$

In case of cytotoxicity, an EC_{50} value (Inhibition concentration 50% relative to the respective vehicle control) was calculated by a linear interpolation method (linear dose-response curve).

3.2 Photo-Irritancy-Factor

For substances which induce a 50 % cytotoxicity (EC_{50}) in the presence and absence of light the Photo-Irritancy-Factor (PIF) is calculated based on comparison of the EC_{50} values in the absence (-UVA) and presence (+UVA) of UVA irradiation.

$$PIF = \frac{EC50\ (-UVA)}{EC50\ (+UVA)}$$ resulting in the following classification rules:

PIF ≥5	phototoxic potential predicted
If 2 < PIF < 5:	probable phototoxic potential predicted
If PIF ≤ 2:	no phototoxic potential predicted

If cytotoxicity occurs only after irradiation a C PIF has to be calculated using the highest test concentration (Cmax) applied in the experimental part in the absence of UV light (-UVA):

$$C\ PIF = \frac{C\max\ (-UVA)}{EC50\ (+UVA)}$$ resulting in the following classification rules:

C PIF > 1	probable phototoxic potential predicted
If C PIF ≤ 1	no phototoxic potential predicted

If no cytotoxicity occurs in the concurrently performed experiments in the absence and presence of UV light up to the highest applied test concentration it has to be considered that the test substance has no phototoxic potential.

In this case, a formal PIF = *1 is used to characterize the result:

$$PIF = *1 = \frac{C\max\ (-UVA)}{C\max\ (+UVA)}$$ resulting in the following classification rule:

PIF = *1	no phototoxic potential predicted

3.3 Mean Photo Effect

The Mean Photo Effect is calculated based on a comparison of the +UVA and -UVA concentration response curves on a grid of concentrations ci (i=1,..., N) chosen from the common concentration range of the (-UVA) and (+UVA) experiments. The photo effect (PEi) at concentration ci is calculated as the product of the concentration effect (CEi) and the response effect (REi). The mean photo effect (MPE) is defined as a weighted averaging across all PEi values, with a weighting factor defined by the highest response value.
The resulting classification rules are:

If MPE ≥ 0.1	phototoxic potential predicted
If MPE < 0.1	no phototoxic potential predicted

For further explanations see Peters & Holzhütter, ATLA 30, 415-432, 2002. A link to a software package for calculation is provided in the Test guideline OECD 432 under http://www.oecd.org/document/55/0,2340,en_2649_34377_2349687_1_1_1_1,00.html.

4. Statistics:

No special statistical tests were performed.
Mean absorbance values and standard deviations were calculated from the single values using calculation software (e.g. MS Excel). The calculations were made using the unedited values. For the report the values were rounded, therefore there may be deviations in the given relative values. Single cell outliers, defined by values that have half or double the value of the respective mean, were excluded from the mean value calculation.

5. Acceptance criteria:

The assay has to be considered valid if the following criteria are met:

- The vehicle control needs to fulfill the following criteria:
  - The mean OD550 value (with and without UVA irradiation) should be > 0.3.
  - CeII viability after irradiation should be at least 80% of the concurrent non-irradiated vehicle control.
  - The standard deviation of the mean values of both vehicle control rows should not exceed ± 15%.
- The positive control chlorpromazine needs to fulfill the following criteria:
  - the EC50 value should be in the ranges:
    With irradiation (+UVA): 0.1 - 2.0 µg/mL
    Without irradiation (-UVA): 7.0 - 90.0 µg/mL
  - and the PIF ≥ 6.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions (see Information in the Amendment).

The stability of the test substance at room temperature in the vehicle DMSO was verified analytically in previous studies (BASF Doc ID 1987/0169-Chromosome Aberration study on Bentazone). The stability in water is given (See Chemical properties in Review Report 7585/VI/97-final).
Osmolarity and pH values were not influenced by test substance treatment. In this study, in the absence and the presence of UVA irradiation no precipitation in culture medium was observed up to the highest applied test substance concentration.

B. CYTOTOXICITY OF THE TEST SUBSTANCE

After treatment with the test substance BAS 351 H (Bentazone) cytotoxic effects indicated by Neutral Red absorbance values of below 50% of control were only observed in the 1st Experiment in the absence of UVA irradiation from 464.2 µg/mL onward (see Table 5.2/4). The 3rd Experiment showed no cytotoxicity with and without UVA treatment.

In the 1st and 3rd Experiment, the absorbance values of the vehicle controls were in the expected range with the accepted variability and, thus, fulfilled the acceptance criteria of this study. The 2nd Experiment was judged invalid due to lacking the predefined criteria of acceptance (data not shown).

According to the results of the present in vitro study, the test substance BAS 351 H (Bentazone) did not lead to a relative reduction in viability of cells exposed in the presence versus absence of light. Therefore, on the basis of these results, the test substance was predicted to have no phototoxic potential (PIF = *1) indicated by Neutral Red Uptake method.

Table 5.2/3 Mean relative cytotoxicity of BAS 351 H with and without UVA irradiation in Balb 3T3 cells

	1st Experiment				3rd Experiment			
	Rel. Cytotoxicity [% of control]	± Rel. Standard deviation [%]	Rel. Cytotoxicity [% of control]	± Rel. Standard deviation [%]	Rel. Cytotoxicity [% of control]	± Rel. Standard deviation [%]	Rel. Cytotoxicity [% of control]	± Rel. Standard deviation [%]
	-UVA		+UVA		-UVA		+UVA	
Vehicle control (1% DMSO)	**100.0**	6.1	**100.0**	5.0	**100.0**	12.3	**100.0**	12.1
BAS 351 H								
4.6 µg/mL	**92.9**	4.4	**85.4**	1.9	**112.3**	3.3	**95.7**	3.1
10.0 µg/mL	**95.2**	8.8	**90.0**	4.7	**116.3**	3.2	**93.6**	6.2
21.5 µg/mL	**90.0**	5.9	**92.1**	3.0	**111.7**	2.3	**94.8**	2.2
46.4 µg/mL	**87.3**	13.7	**88.2**	6.4	**110.1**	3.2	**86.4**	2.6
100.0 µg/mL	**75.3**	10.2	**79.9**	1.7	**110.7**	4.6	**89.5**	4.8
215.4 µg/mL	**66.5**	18.5	**72.9**	3.4	**107.7**	4.2	**79.4**	2.5
464.2 µg/mL	**41.3**	8.2	**81.0**	4.2	**104.4**	2.9	**81.3**	9.3
1000.0 µg/mL	**40.7**	2.6	**76.0**	10.7	**100.9**	6.1	**66.0**	18.9

C. CYTOTOXICITY OF THE POSITIVE CONTROL

For the positive control substance Chlorpromazine a concentration-dependent decrease in the Neutral Red absorbance values in the absence and the presence of UVA irradiation was in the expected range with the accepted variability and, thus, fulfilled the acceptance criteria of this study. The EC_{50} values were found to be 45.6 and 45.3 µg/mL without UVA irradiation and 0.7 and 0.6 µg/mL with UVA irradiation in the 1st and 3rd Experiment, respectively (see Table 5.2/4). Based on these observations a PIF of 63.2 in the 1st Experiment and a PIFof 74.9 in the 3rd Experiment was calculated. Based on these values, the sensitivity of the test method with and without UVA irradiation was clearly demonstrated. The values were in the expected range with the accepted variability and, thus, fulfilled the acceptance criteria of this study.

Table 5.2/4 Mean relative cytotoxicity of Chlorpromazine with and without UVA irradiation in Balb/c 3T3 cells

	1st Experiment				3rd Experiment			
	Rel. Cytotoxicity [% of contro]	± Rel. Standard deviation [%]	Rel. Cytotoxicity [% of control]	± Rel. Standard deviation [%]	Rel. Cytotoxicity [% of control]	± Rel. Standard deviation [%]	Rel. Cytotoxicity [% of control]	± Rel. Standard deviation [%]
	-UVA		+UVA		-UVA		+UVA	
Vehicle control (1% DMSO)	**100.0**	4.7	**100.0**	14.2	**100.0**	11.8	**100.0**	7.6
Chlorpromazine								
0.03 µg/mL	**-**	-	**107.5**	8.5			**95.9**	12.3
0.05 µg/mL	**-**	-	**81.8**	19.8			**98.1**	16.2
0.1 µg/mL	**-**	-	**105.1**	6.7			**99.9**	8.0
0.2 µg/mL	**-**	-	**97.2**	10.5			**86.4**	10.4
0.4 µg/mL	**-**	-	**73.7**	20.3			**85.4**	12.2
0.8 µg/mL	**-**	-	**44.3**	10.9			**16.2**	10.4
1.6 µg/mL	**-**	-	**14.3**	2.6			**0.0**	0.2
1.9 µg/mL	**102.1**	2.3			**104.4**	7.0		
3.2 µg/mL	**-**	-	**-0.1**	0.6			**1.4**	0.4
3.8 µg/mL	**101.5**	6.3			**105.8**	4.1		
7.5 µg/mL	**98.5**	3.5			**103.7**	7.0		
15.0 µg/mL	**90.0**	5.8			**99.3**	13.0		
30.0 µg/mL	**86.9**	7.4			**88.3**	15.4		
60.0 µg/mL	**16.1**	8.2			**13.1**	7.5		
90.0 µg/mL	**0.5**	0.2			**-0.2**	0.2		
180.0 µg/mL	**0.1**	0.1			**0.0**	0.2		

III. CONCLUSIONS

According to the results of the present study, the test substance BAS 351 H (Bentazone) is considered **not to be a phototoxic substance** in the in vitro 3T3 NRU Phototoxicity Test using Balb/c 3T3 cells under the experimental conditions chosen here.

5.3 Short-term toxicity

Studies presented in the original Annex II Dossier (1995): Short-term toxicity studies (28 - 90 days) with oral administration are available from three different species (rats, mice, dogs). A one-year oral dog study was carried out. Furthermore, three 21-day dermal toxicity studies were performed in rabbits. These studies have been evaluated by European authorities and Germany as Rapporteur member state in 1998 (European Commission Peer Review Program, BASF Doc ID 1998/1001178) and were considered to be acceptable. For the convenience of the reviewer, these are listed in Table 5.3/1.

Subacute and subchronic feeding studies were carried out in rats, mice and dogs with Bentazone as free acid. The NOEL in a 4-week oral study in rats was approximately 200 mg/kg bw/d based on hemorrhages in kidneys and ovaries at around 500 mg/kg bw. A NOEL for the subacute mouse study could not be established. In the most recent 90-day rat study, a NOEL of about 25 (males) or 29 (females) mg/kg bw/d was obtained confirming previous results. In the 90-day study in dogs, the NOEL was approximately 12 mg/kg bw/d with mortalities and signs of severe intoxication at the highest dose level of 3,000 ppm (about 114 mg/kg bw/d) suggesting that the maximal tolerated dose (MTD) was already exceeded. In a more recent one-year study in dogs, a similar NOEL of about 13.1 mg/kg bw/d was established.
Short-term dermal administration of bentazone up to 1,000 mg/kg bw did not reveal any adverse effects in two valid studies in rabbits. A third one confirmed this result but was considered unacceptable due to infections.

Table 5.3/1 Summary of peer-reviewed subacute and subchronic toxicity studies performed with BAS 351 H

Type of study (strain)	Test substance Dose level	Result / NOAEL	Reference
28-day oral route – rat (Fischer 344)	BAS 351 H (acid) 0, 600 ; 1,800 ; 5,000 ; 10,000 ppm	10,000 ppm: mortality, reduced bw and bw gain ; changed hematological parameters, hemorrhages in various tissues, increased weight of liver and kidneys decreased weight of heart and testicles. 5,000ppm (actually found to be 4000 ppm): hemorrhages in kidneys and ovaries. **1,800 ppm (= 200 mg/kg bw/day): NOEL**	[redacted] 1981(a) DocID 1981/10240
28-day oral route – mice (B6C3F1)	BAS 351 H (acid) 0 ; 400 ; 2,000 ; 5,000 and 10,000 ppm	5,000 and 10,000 ppm: up to 100 % mortality, hemorrhages in various tissues. 2,000 ppm: changed coagulation parameters. **no NOEL**	Anonymous, 1981a DocID 1981/10239 [redacted] 1982 DocID 1982/1000611
90-day oral route – rat (Sprague-Dawley)	BAS 351 H (acid) 0 ; 70 ; 200 ; 800 ; 1,600 ppm	1,600 ppm : retarded bw gain ; increased relative kidney weight. 800 ppm: increased relative kidney weight in females. **200 ppm (about 10 mg/kg bw/day): NOEL**	[redacted] 1970(a) DocID 1970/008
90-day oral route - rat (Wistar)	BAS 351 H (acid) 0 ; 400 ; 1,200 ; 3,600 ppm	3,600 ppm : mortality (4/10 males) ; retarded bw gain in females ; changed hematological and clinicochemical parameters. 1,200ppm: changed clinico-chemical parameters in males **400 ppm (= 25 / 29 mg/kg bw/day m/f): NOEL**	[redacted] 1987 DocID 1987/0173
90-day oral route – dog (Beagle)	BAS 351 H (acid) 0 ; 100 ; 300 ; 1,000 ; 3,000 ppm	6,000 ppm: mortality (3/6 animals); sedation; hemorrhage conjunctivitis; ulcerative stomatitis (males); changed haematological and clinicochemical parameters; increased relative and absolute liver and kidney weights. 1,000 ppm: sedation (1/6 animals) **300 ppm (= 12 mg/kg bw/day): NOEL**	[redacted] 1970 DocID 1970/009 [redacted] 1973(a) DocID 1973/0057 [redacted] 1972(a) DocID 1972/0061
52-week oral route – dog (Beagle)	BAS 351 H (acid) 0 ; 100 ; 400 ; 1,600 ; ppm	1,600 ppm: transient decrease in bw; changes in hematological parameters. **400 ppm (= 13.1 mg/kg bw/day): NOEL**	II A 5.3.4/1 [redacted] 1989(a) DocID 1989/0049 II A 5.3.4/2 [redacted] 1989(a) DocID 1989/0153

Table 5.3/1 Summary of peer-reviewed subacute and subchronic toxicity studies performed with BAS 351 H

Type of study (strain)	Test substance Dose level	Result / NOAEL	Reference
21-day dermal toxicity – rabbit (New Zealand)	BAS 351 H (acid) 0; 250; 500; 1,000 mg/kg bw	**No systemic or local toxicity**	II A 5.3.7/1 [redacted] 1971(a) DocID 1971/005
21 day dermal toxicity – rabbit	BAS 351 H (acid) 0; 250; 500; 1,000 mg/kg bw	Excluded from evaluation due to infection with coccidian.	II A 5.3.7/2 [redacted] 1988(a) DocID 1988/0350
21-day dermal toxicity – rabbit (New Zealand)	BAS 351 H (acid) 0; 250; 500; 1,000 mg/kg bw	**No systemic or local toxicity**	II A 5.3.7/3 [redacted] 93(a) DocID 1993/10760

According to these studies**,** the predominant target of Bentazone (as free acid) after short-term to subchronic oral exposure of rats, mice and dogs is the blood coagulation system (prolonged blood coagulation time with subsequent haemorrhages at higher dose levels). Clinical chemistry changes (without specific pattern) and increased urinary output as well as increased kidney weights suggested that the kidney and to a lesser extent the liver are additional target organs of minor sensitivity, albeit there were no histopathological changes at any dose level. Body weight reduction was induced in all species at higher dose levels. The comparison of the NOELs has shown that interspecies variation regarding bentazone is rather low.

Submission of not yet peer-reviewed studies in this AIR 2-Dossier:
For Bentazone-sodium, which is the manufactured use product, the check regarding toxicological equivalence to Bentazone-acid was outstanding and has been done in a comparative 13-week oral toxicity (feeding) study in Wistar rats. The comparison of Bentazone-sodium and Bentazone-acid in Wistar rats over a period of 3 months after oral administration revealed similar signs of toxicity for both compounds at the high equimolar dose. Furthermore, the findings were well comparable with the findings of the former subchronic study (see Monograph, Vol.3, Annex B-5, September 1996, chapter 5.3.2.2, BASF DocID 1987/0173), although the no observed effect levels seem to be different, with Bentazone-acid showing effects at 1200 ppm versus no effect on clinicochemical parameters seen at 1425 ppm with Bentazone-sodium. These differences are considered to reflect the biological variation within the animals.

Table 5.3/2 Subchronic toxicity study with BAS 351 H for evaluation in the AIR II process

Type of study (strain)	**Test substance (batch / purity) Dose level**	**Result / NOAEL**	**Reference**
90-day oral route - rat (Wistar)	BAS 351 H (COD-001416/100%) 3,600 ppm BAS 351 H-Na (COD-001417/91.9%) 0-475-1,425-4,275 ppm	3,600 ppm BAS 351 H: increased kidney (abs. &rel.) and liver (rel.) weights in females; changed hematological parameters in males; changed clinicochemical and urinary parameters 4,275 ppm BAS 351 H-Na: increased kidney (abs. &rel.) and liver (rel.) weights in females; changed hematological parameters in males; changed clinicochemical parameters **1,425 ppm** BAS 351 H-Na **(= 91 / 98 mg/kg bw/day m/f): NOAEL**	AIR2-Dossier II A 5.3.2/1 [redacted] 2011 DocID 2011/1173365 AIR2-Dossier II A 5.3.2/2 [redacted] 2012 DocID 2012/1009658

In summary, no classification for repeated exposure, like specific target organ toxicity, is considered necessary for Bentazone as free acid, BAS 351 H, as the guidance values and the criteria were not met. The manufactured use product Bentazone-sodium can be regarded as equivalent to Bentazone-acid after oral administration on an equimolar base.
The agreed endpoint for derivation of the AOEL (oral exposure) was based on the NO(A)EL in the 1-year dog study, which was comparable to that of the 90 day dog study. The NOEL in an older 90-day rat study was slightly lower, however, the more recent rat study revealed a higher value. Thus, it was reasonable to consider the dog as the most sensitive species to bentazone-related toxicity. Since no inhalation toxicity was observed in the acute studies, a study with repeated administration had not been conducted. An AOEL for inhalation was agreed to be not relevant. A dermal AOEL was established on the basis of the most recent 21-day dermal study in rabbits.

Therefore, the endpoints fixed in the European Commission Review report for the active substance bentazone (7585/VI/97-final from 30 November 2000) regarding short term toxicity and AOEL Reference values are considered to be still valid.

EU agreed Endpoints for bentazone (EC Review report, 2000)

Short term toxicity

Target / critical effect:	Blood (prolonged blood coagulation time, diminished hematocrit , reduced haemoglobin); liver and kidney
Lowest relevant oral NOAEL / NOEL:	400 ppm (13 mg/kg bw/d), 1 year dog study,
Lowest relevant dermal NOAEL /NOEL:	1000 mg/kg bw/d, (21-day rabbit dermal study)
Lowest relevant inhalation NOAEL /NOEL:	not required

AOEL derivation

AOEL systemic	0.13 mg/kg bw/d (based on 1-year dog study and a Safety factor of x100)
AOEL inhalation	not relevant

5.3.1 Oral 28-day toxicity

No new studies were performed. All information available is presented in the original Annex II Dossier (1995) and has been evaluated by European authorities and Germany as Rapporteur member state.

5.3.2 Oral 90-day toxicity (rodents)

Report: II A 5.3.2/1
[redacted] 2011(a)
Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) and Reg.No. 51929 (Bentazone-acid, BAS 351 H) - A comparative repeated dose 90-day oral toxicity study in Wistar rats - Administration via the diet
BASF DocID 2011/1173365

Date of report: 16-Dec-2011

Testing facility: [redacted]

Guidelines: OECD 408; EPA 870.3100; (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.26

GLP: Yes
(laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report: II A 5.3.2/2
[redacted] 2012(a)
Amendment No. 1: Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) and Reg.No. 51929 (Bentazone-acid, BAS 351 H) - A comparative repeated dose 90-day oral toxicity study in Wistar rats - Administration via the diet
BASF DocID 2012/1009658

Date of report: 13-Jan-2012

Testing facility: [redacted]

Guidelines: OECD 408; EPA 870.3100; (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.26

GLP: Yes
(laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Note: The Amendment provides corrected incidence data for the parameter "heart inflammation, myocard" in the pathology report which was correctly documented in the individual data with 1 male animal in group 4 but was by mistake documented in the Incidence table IC 19/22 with 4 male animals. The main part of the original report is not affected.

Executive Summary

Dietary administration of Reg. No. 88691 (Bentazone-sodium; Batch: COD-001417, Purity: 91.9%) to Wistar rats at dose levels of 0, 475, 1425 ppm and 4275 ppm resulted in the main in the typical pattern of effects:

4275 ppm Bentazone-sodium

- prolonged activated partial thromboplastin time (PTT) and prothrombin time (QT) in males.
- decreased globulin values in females.
- increased kidney (absolute and relative) weights and relative liver weights in females without histopathological correlate. These organ weight increases were considered to be caused by increased water consumption in females towards the end of the administration period.
- There was also a trend to reduced body weights in both sexes, with a body weight of 6.6% / 3.7% below control and a body weight gain reduction about 10.6/10.5% towards the end of the study in males/females, respectively.

1425 ppm Bentazone-sodium showed no treatment-related adverse effect. Only a slight substance-induced exacerbation of the general occurring chronic nephropathy was found in male rats, which was not considered as adverse as the severity was not increased over the grading already found in control animals.

For comparison with the high dose Bentazone-sodium group an equimolar dosed group of Reg. No. 51929 (Bentazone-acid, BAS 351 H), which is 3600 ppm, was administered in parallel to 10 male and 10 female rats. This equimolar Bentazone-acid group showed comparable results than Bentazone-sodium:

3600 ppm Bentazone-acid

- prolonged activated partial thromboplastin time (PTT) and prothrombin time (QT) in males.
- decreased globulin values in females.
- increased kidney (absolute and relative) weights and relative liver weights in females without histopathological correlate. These organ weight increases were considered to be caused by increased water consumption in females towards the end of the administration period.
- The trend to reduced body weights in both sexes reached differences in body weight of 4.5% / 4.2% below control and a body weight gain reduction about 7.7/10.7% towards the end of the study in males/females, respectively.

Beside this, also cholesterol, triglyceride and potassium values were slightly increased in females.

In conclusion, the comparison of Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na) and Reg. No. 51929 (Bentazone-acid, BAS 351 H) in Wistar rats over a period of 3 months oral administration revealed similar signs of toxicity for both compounds at the high equimolar dose tested. Furthermore, the findings were well comparable with the findings of the former subchronic study (see Monograph, Vol.3, Annex B-5, September 1996, chapter 5.3.2.2 BASF DocID 1987/0173).

Based on the clinical chemistry and the organ weight changes observed at 4275 ppm with Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na), a NOAEL of 1425 ppm was identified in this study, which is equivalent to mean daily doses of about 91 mg/kg bw/d in males and 98 mg/kg bw/d in females.

(DocID 2011/1173365)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

a) Sodium salt form:	Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na)
Description:	solid/yellowish
Lot/Batch #:	COD-001417
Purity:	91.9% (tolerance ±1.0%)
Stability of test compound:	The test substance was stable over the study period (Expiry date 01.11.2013).

b) Free Acid form:	Reg. No. 51929 (Bentazone-acid, BAS 351 H)
Description:	solid/yellowish
Lot/Batch #:	COD-001416
Purity:	100.0% (tolerance ±1.0%)
Stability of test compound:	The test substance was stable over the study period (Expiry date 01.11.2013).

2. Vehicle and/or positive control: Rodent diet

3. Test animals:

Species:	Rat
Strain:	Crl:WI (Han)
Sex:	Male and female
Age:	32 ± 1 days at delivery; approx. 42 ± 1 days at start of administration
Weight at dosing:	Weight variation ≤ 20 % of mean: ♂: 152.6-189.8 g, ♀ 119.7-145.4 g
Source:	Charles River, Germany
Acclimation period:	10 days
Diet:	Kliba maintenance diet for mouse/rats “GLP”, Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	Tap water in bottles, ad libitum
Housing:	Group housing (5 animals/cage) housed in H-Temp (PSU) cages (TECNIPLAST Deutschland GmbH, Hohenpreißenberg, Germany, floor area about 2065 cm² dust free embedding). Enrichment material: wooden gnawing blocks (Typ NGM E-022, Abedd® Lab. and Vet. Service GmbH, Vienna, Austria) Motor activity measurements were conducted in Polycarbonate cages with wire covers from Ehret, Emmendingen (floor area about 800 cm²) and small amounts of absorbent material
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	15/hour
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 02-March-2011 - 16-November-2011
(In life dates: 18-March (start of administration) to 20-22-June-2006 (blood sampling and necropsy))

2. Animal assignment and treatment:
Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na) was administered to groups of 10 male and 10 female Wistar rats at dietary concentrations of 0 (test group 0, control), 475 (test group 1, low dose), 1425 ppm (test group 2, mid dose) and 4275 ppm (test group 3, high dose) for at least 90 days. For comparison an equimolar dose of 3600 ppm (test group 4) of Reg. No. 51929 (Bentazone-acid, BAS 351 H) was also administered via the diet over a period of 3 months.

The animals were assigned to the treatment groups by means of computer generated randomization lists based on body weights.

3. Test substance preparation and analysis:

For each concentration of Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na) an appropriate solution in acetone was prepared. This solution was sprayed on about 3 kilogram food under partial vacuum in a laboratory evaporator. Subsequently acetone was removed from this premix at a temperature of about 40°C for about 60 minutes.
For the concentration of Reg. No. 51929 (Bentazone-acid, BAS 351 H) the test substance was weighed out and mixed with a small amount of food to generate the premix.

Analyses performed prior to the start of the administration period revealed that the test-substance Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na) was stable in the diet for at least 32 days whereas Reg. No. 51929 (Bentazone-acid, BAS 351 H) was stable in the diet over a period of at least 49 days. As the mixtures were stored no longer than this time period, the stability was guaranteed.

Homogeneity and concentration analyses of the diet preparations were performed at the beginning and at the end of administration for all concentrations. For this purpose, three randomly sampled specimen were analyzed. No test-article was determined in control diets.

Reg. No. 88691 (Bentazone-sodium): Analysis of diet preparations for homogeneity and test-item content

Dose level [ppm]	Sample-No.	Sampling	Analysis	Concentration [ppm] Mean ± SD#	Relative standard deviation [%]	Mean % of nominal concentration
0 ppm	2	17.03.11	12.&13.04.11	n.d.	---	---
	17	09.06.11	22.07.11	n.d.	---	---
			Average	n.d.	---	---
475 ppm	3	17.03.11-	12.&13.04.11	463.7 ± 2.4	0.5	97.6
	4			477.2 ± 0.3	0.1	100.5
	5			487.8 ± 2.3	0.5	102.7
			Average	**476.2**	**± 2.5**	**100.3**
	18	09.06.11	22.07.11	465.6		98.0
	19			403.2		84.9
	20			421.7		88.8
			Average	**430.2**	**± 7.4**	**90.6**
1425 ppm	6	17.03.11-	12.&13.04.11	1427.5 ± 4.9	0.3	100.2
	7			1393.5 ± 3.5	0.2	97.8
	8			1413.0 ± 2.8	0.2	99.2
			Average	**1411.3**	**± 1.2**	**99.0**
	21	09.06.11	22.07.11	1340		97.0
	22			1417		99.4
	23			1361		95.5
			Average	**1372.7**	**± 2.9**	**96.3**
4275 ppm	9	17.03.11-	12.&13.04.11	4239.0 ± 7.1	0.2	99.2
	10			4414.0 ± 1.4	0.0	103.3
	11			4324.0 ± 9.9	0.2	101.1
			Average	**4325.7**	**± 2.0**	**101.2**
	24	09.06.11	22.07.11	4436		103.8
	25			4291		100.4
	26			4289		100.3
			Average	**4338.7**	**± 1.9**	**101.5**

n.d.: not detectable; # SD based on two individual analytical measurements
Values may not calculate exactly due to rounding of values

The relative standard deviation of the homogeneity samples ranged between 1.2 and 2.9 % except for the samples 18-20 (low dose samples) prepared on June 09, 2011 which were with 7.4% slightly above the 5 % limit described in the SOP. Nevertheless, the low relative standard deviations in the homogeneity analysis indicate the homogenous distribution of Reg. No. 88691 (Bentazone-sodium) in the diet preparations, which are in general regarded acceptable for diet analysis if below 10%. The actual (mean) average test-substance concentrations were in the range of 90.6 to 101.5% of the nominal concentrations, thereby demonstrating the correctness of the concentrations in the diet.

The relative standard deviations of the homogeneity samples of Reg. No. 51929 (Bentazone-acid, BAS 351 H) ranged between 0.7 and 1.3%, thereby indicate the homogenous distribution of the test-item in the diet preparations. The actual (mean) average test-substance concentrations were two times 97.1 of the nominal concentrations, thereby demonstrating the correctness of the concentrations of the test-item in the diet.

Reg. No. 51929 (Bentazone-acid, BAS 351 H)): Analysis of diet preparations for homogeneity and test-item content

Dose level [ppm]	Sample-No.	Sampling	Analysis	Concentration [ppm] Mean ± SD#	Relative standard deviation [%]	Mean % of nominal concentration
0 ppm	2	18.03.11	12.04.11	n.d.	---	---
	17	09.06.11	22.07.11	n.d.	---	---
			Average	n.d.	---	---
3600 ppm	3	18.03.11	12.04.11	3442.0 ± 8.5	0.2	95.6
	4			3528.0 ± 5.6	0.2	98.0
	5			3516.5 ± 2.1	0.1	97.7
			Average	**3495.5**	**± 1.3**	**97.1**
	18	09.06.11	22.07.11	3496		97.1
	19			3524		97.9
	20			3472		96.4
			Average	**3497.3**	**± 0.7**	**97.1**

n.d.: not detectable; # SD based on two individual analytical measurements
Values may not calculate exactly due to rounding of values

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
body weight, body weight change	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means
feces, rearing, grip strength forelimbs, grip strength hindlimbs, foot-splay test, motor activity	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians

Statistics of clinical pathology

Parameter	Statistical test
Clinical pathology parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using Wilcoxon-test (two-sided) for the equal medians

Statistics of pathology

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using WILCOXON-test (two-sided) for the equal medians

C. Methods

1. Observations:

The animals were examined for morbidity or mortality twice daily on working days and once daily on weekends and public holidays. Animals in moribund stage were sacrificed under isoflurane anesthesia and necropsied. Observations for overt clinical signs of toxicity were performed at least once daily.
Detailed clinical observations were performed in all animals prior to the start of the administration period and weekly thereafter. For this, the animals were transferred to a standard arena (50 x 37.5 cm with sides of 25 cm high). The findings were ranked according to the degree of severity, if applicable. The clinical examination included, but was not limited, to the following parameters/organs:

1. abnormal behavior during handling	10. abnormal movements
2. fur	11. impairment of gait
3. skin	12. lacrimation
4. body posture	13. palpebral closure
5. salivation	14. exophthalmus
6. respiration	15. feces (appearance/consistency)
7. activity/arousal level	16. urine
8. tremors	17. pupil size
9. convulsions	

2. Ophthalmoscopy:

Prior to the start of the administration period the eyes of all animals, and on day 91 the eyes of the control and high dose animals were examined for any changes using an ophthalmoscope after administration of a mydriatic.

3. Body weight:

The body weight of the animals was determined before the start of the administration period (in order to randomize the animals), at the start of the treatment (day 0), and once weekly thereafter. The difference between the body weight on the respective day of weighing and the body weight on study day 0 was calculated as body weight change.

4. Food consumption and compound intake:

Food consumption was determined once weekly on a cage basis and calculated as mean food consumption in grams per animal and day.

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

with FC_x as the mean daily food consumption (in g/day) on day x, C as the concentration of test substance in the food in ppm and BW_x as body weight on day x (in g).

5. Water consumption:

Group water consumption was determined weekly for each cage. The average water consumption per cage was used to estimate the mean water consumption in grams per animal per day.

6. Functional observation battery (FOB):

A functional observational battery was performed in all animals in a randomized order at the end of the administration period starting at about 09:30-10:00 h a.m. For this purpose, the animals were placed individually in polycarbonate cages during the time of the examination. The FOB started with passive observations without disturbing the animals (home cage observations), followed by removal from the home cage and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. The findings were ranked according to the degree of severity, if applicable (for details see Part III of the report).

During the home cage observation, special attention was paid to posture, tremors, convulsions, abnormal movements, impairment of gait and other findings.

For open field observation, the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

1. behavior when removed from cage	10. respiration
2. fur	11. tremors
3. skin	12. convulsions
4. salivation	13. abnormal movements
5. nose discharge	14. impairment of gait
6. lacrimation	15. activity/arousal level
7. eyes/pupil size	16. feces (number of fecal pellets/appearance/ consistency) within two minutes
8. posture	17. urine (appearance/quantity) within two minutes
9. palpebral closure	18. number of rearings within two minutes

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

1. approach response	8. behavior during "handling"
2. touch response	9. vocalization
3. vision ("visual placing response")	10. pain perception ("tail pinch")
4. pupillary reflex	11. grip strength of forelimbs
5. pinna reflex	12. grip strength of hind limbs
6. audition ("startle response")	13. landing foot-splay test
7. coordination of movements ("righting response")	14. other findings

7. Motor activity measurement:

Motor activity was measured on the same day the FOB was performed. The examinations were performed using the TSE Labmaster System supplied by TSE Systems GmbH, Bad Homburg, Germany with 18 beams per cage. For this measurement the rats were placed in clean Polycarbonate cages with small amounts of bedding material in random sequence. Motor activity measurements were performed from 2.00 p.m. onwards. The number of beam interrupts was counted over twelve 5-minute intervals. For each rat, measurement was started individually when the 1st beam was interrupted (measuring variant "staggered start") and finished exactly 1 hour later. During the measurements, the animals received no food and no water. After the transfer of the last rat in each case, the room of measurement was darkened.

8. Estrous cycle determination:

Vaginal smears for individual estrus cycle determination were prepared in the morning of the day of sacrifice according to the timetable and evaluated. The samples were disposed after examination. The differentiation was conducted according to following stages:

Code	Cycle stage	Appearance in vaginal smear
1	Diestrous	leucocytes, few nucleated epithelial cells
2	Proestrous	single leucocytes, many nucleated and few horny epithelial cells
3	Estrous	only horny epithelial cells
4	Metestrous	leucocytes, some horny epithelial cells and some nucleated epithelial cells

9. Hematology and clinical chemistry:

Blood was withdrawn in the morning from fasted, isoflurane-anesthetized animals from the retro-orbital plexus. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence.

The following hematological and clinical chemistry parameters were determined for all animals:

Hematology:					
	Red blood cells		*White blood cells*		*Clotting Potential*
✓	Erythrocyte count (RBC)	✓	Total leukocyte count (WBC)	✓	Prothrombin time (Quick's test, QT)
✓	Hemoglobin (Hb)	✓	Neutrophils (differential)	✓	Thrombocyte count
✓	Hematocrit (Hct)	✓	Eosinophils (differential)		Activated partial thromboplastin time (PPT)
✓	Mean corp. volume (MCV)	✓	Basophils (differential)		
✓	Mean corp. hemoglobin (MCH)	✓	Lymphocytes (differential)		
✓	Mean corp. Hb. conc. (MCHC)	✓	Monocytes (differential)		
✓	Reticulocytes				
Clinical chemistry:					
	Electrolytes		*Metabolites and Proteins*		*Enzymes:*
✓	Calcium	✓	Albumin	✓	Alanine aminotransferase (ALT)
✓	Chloride	✓	Bilirubin (total)	✓	Aspartate aminotransferase (AST)
✓	Magnesium	✓	Cholesterol	✓	Alkaline phosphatase (ALP)
✓	Phosphorus (inorganic)	✓	Creatinine	✓	□-glutamyl transpeptidase (GGT)
✓	Potassium	✓	Globulin (by calculation)		
✓	Sodium	✓	Glucose		
		✓	Protein (total)		
		✓	Triglycerides		
		✓	Urea		

10. Urinalysis:

For urinalysis, individual animals were transferred to metabolism cages and urine was collected overnight. No food or water was supplied during urine collection. The samples were analyzed in a randomized order.

The following parameters were determined for all animals:

Urinalysis					
	Quantitative parameters:		*Semiquantitative parameters*		
✓	Urine volume	✓	Bilirubin	✓	Protein
✓	Specific gravity	✓	Blood	✓	pH-value
		✓	Color and turbidity	✓	Urobilinogen
		✓	Glucose	✓	Sediment (microscopical exam.)
		✓	Ketones		

11. Sacrifice and pathology:

The animals were sacrificed by decapitation under Isoflurane anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology.
The following organs were sampled, weighed and examined histopathologically:

Pathology:

The following organs were collected (column C), weighed (W) and examined histopathologically (H, ✓: all groups, #: control and test group 3 and 4).

C	W	H		C	W	H		C	W	H	
✓	✓	#	adrenals	✓	✓	✓	kidneys[&]	✓		#	seminal vesicles
✓		#	aorta	✓			lachrymal glands[%]	✓		#	skin
✓		#	bone marrow[§]	✓		#	larynx	✓		#	spinal cord (3 levels)[@]
✓	✓	#	brain	✓	✓	#	liver	✓	✓	#	spleen
✓		#	caecum	✓		#	lung	✓			sternum w. marrow
✓		#	coagulation glands	✓		#	lymph nodes[#]	✓		#	stomach (fore- & glandular)
✓		#	colon	✓		#	mammary gland (♀)	✓	✓	#	testes
✓		#	duodenum	✓			muscle, skeletal	✓	✓	#	thymus
✓	✓	#	epididymides	✓		#	nerve, peripheral (sciatic n.)	✓	✓	#	thyroid/parathyroid
✓		#	esophagus	✓		#	nose/nasal cavity[‡]	✓		#	trachea
✓		#	eyes (with optic nerve)	✓	✓	#	ovaries and oviduct**	✓		#	urinary bladder
✓		#	femur (with joint)	✓		#	pancreas	✓	✓	#	Uterus with cervix
✓		✓	gross lesions	✓		#	pharynx	✓		#	vagina
✓			harderian gland	✓		#	pituitary				
✓	✓	#	heart	✓		#	prostate		✓		body (anesthetized animals)
✓		#	ileum	✓		#	rectum				
✓		#	jejunum (w. Peyer's patches)	✓		#	salivary glands*				

[§] from femur; [#] axillary and mesenteric; [@] cervical, thoracic, lumbar; *mandibular and sublingual, ** oviduct not weighed; [%] extraorbital, [‡] histopathology at level III, [&] histopathological evaluation of all treatment groups for males only

The organs or tissues were fixed in 4% buffered formaldehyde solution or in modified Davidson's solution.

After completion of the histopathological assessment by the study pathologist an internal peer review was performed including all kidneys of male animals. Results presented reflect the consensus opinion of the study pathologist and the peer review pathologist.

II. RESULTS AND DISCUSSION

A. TEST SUBSTANCE ANALYSES

See Section B 3. above.

B. OBSERVATIONS

1. Clinical signs of toxicity

No test substance-related findings were observed.

2. Mortality

In the present study no animal died ahead of schedule.

3. Ophthalmoscopy

No treatment-related ophthalmologic findings were noted, either with Reg No. 88691 (Bentazone-sodium, BAS 351 H-Na) or with Reg. No. 51929 (Bentazone-acid, BAS 351 H). The only findings observed at the end of the treatment period were corneal stipplings and remainders of the papillary membrane in single animals lacking a dose-response relationship. The incidence of these findings was either comparable between control and high dose groups or already observed in the groups prior to commencement of treatment.

C. FOB and MOTOR ACTIVITY

Neither home cage nor open field observations revealed any indication of treatment-related effects. The same holds true for the sensorimotor tests and reflexes as well as for the quantitative parameters. The following tables list the observed findings with deviations from rank "zero value":

1. Quantitative parameters

No statistically significant differences for defecation, number of rearings, fore- and hind limb grip strength or foot splay width were observed in any treated group.

2. Home cage observations

Deviations from (rank) "zero values" were obtained in several animals [see Table 5.3/3]. However, as all findings were equally distributed between treated groups and controls, deviations were considered to be incidental. Parameters investigated included posture, tremors, convulsions, abnormal movements and impairment of gait.

Table 5.3/3 Home cage observations with deviations from rank zero

Sex (number of animals)		Males (N=10)					Females (N=10)				
Test-item	Rank		BAS 351 H-Na			BAS 351 H		BAS 351 H-Na			BAS 351 H
Dose [ppm]		0	475	1425	4275	3600	0	475	1425	4275	3600
Posture											
- animal is sitting or lying	0	4	5	3	4	3	5	2	3	2	5
- animal is staying or moving	1	6	5	7	6	7	5	8	7	8	5
Impairment of gait											
-animal is not walking during observation	0	4	5	3	4	1	5	2	3	2	3
-no impairment of gait	1	6	5	7	6	9	5	8	7	8	7

3. Open-field observations

Deviations from "zero values" were obtained in several animals [see Table 5.3/4]. However, as all findings were equally distributed between treated groups and controls, these observations were considered incidental.

Table 5.3/4 Open field observations with deviations from rank zero

Sex (number of animals)		Males (N=10)					Females (N=10)				
Test-item	Rank		BAS 351 H-Na			BAS 351 H		BAS 351 H-Na			BAS 351 H
Dose [ppm]		0	475	1425	4275	3600	0	475	1425	4275	3600
Urine											
- no urination during observation period	0	6	7	9	7	7	10	9	7	9	10
- urine without abnormalities	1	4	2	1	3	3	0	1	3	1	0
Posture											
- animal is sitting or lying	0	0	0	0	0	0	0	0	0	0	0
- animal is staying or moving	1	10	10	10	10	10	10	10	10	10	10
Impairment of gait											
-animal is not walking during observation	0	0	0	0	0	0	0	0	0	0	0
-no impairment of gait	1	10	10	10	10	10	10	10	10	10	10

4. Sensorimotor tests / reflexes

Deviations from rank "zero values" were obtained in several animals [see Table 5.3/5]. However, as all findings were equally distributed between treated groups and controls, these observations were considered incidental.

Table 5.3/5 Sensorimotor tests / reflexes with deviations from rank zero

Sex (number of animals)		Males (N=10)					Females (N=10)				
Test-item	Rank		BAS 351 H-Na			BAS 351 H		BAS 351 H-Na			BAS 351 H
Dose [ppm]		0	475	1425	4275	3600	0	475	1425	4275	3600
Approach response											
- no reaction	0	0	1	0	2	1	6	3	6	5	3
- approaching to object	1	10	9	10	8	9	4	7	4	5	7
Touch response											
- no reaction	0	6	5	6	8	7	8	8	9	10	10
- orientation to the stimulus	1	4	5	4	2	3	2	2	1	0	0

5. Motor activity

Regarding the overall motor activity as well as single intervals, no test substance-related deviations were noted for male and female rats.

6. Conclusion on FOB and Motoractivity

In conclusion, both Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na) and Reg. No. 51929 (Bentazone-acid, BAS 351 H) are comparable and induce no statistically significant effects in Wistar rats at equimolar dietary dose levels after oral administration over a period of 3 months in regard to FOB- and motor activity-testing. This result confirms the results of the 90-day oral rat neurotoxicity study with Bentazone (BAS 351 H) [see chapter 5.7.4, BASF Doc ID 2004/1013171 and 2004/1025741].

D. BODY WEIGHT AND BODY WEIGHT GAIN

Although no significant changes in body weight were measured in treated male and female animals compared to controls, there was a trend to reduced body weights in both sexes of group 3 (4275 ppm; Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na)) and group 4 (3600 ppm; Reg. No. 51929 (Bentazone-acid, BAS 351 H)) towards the end of the study. The values on day 91 were 6.6/3.7% [males/females] below control in group 3 (Bentazone-sodium treatment) and 4.5/4.2% below control in group 4 (bentazone-acid treatment). Corresponding body weight change values were 10.6/10.5% below control in group 3 and 7.7/10.7% below control in group 4.

No treatment-related effects on body weight were noted at dose levels ≤ 1425 ppm Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na).

Figure 5.3/ 1 Body weight development of rats administered Bentazone as sodium-salt or free acid for at least 90 days

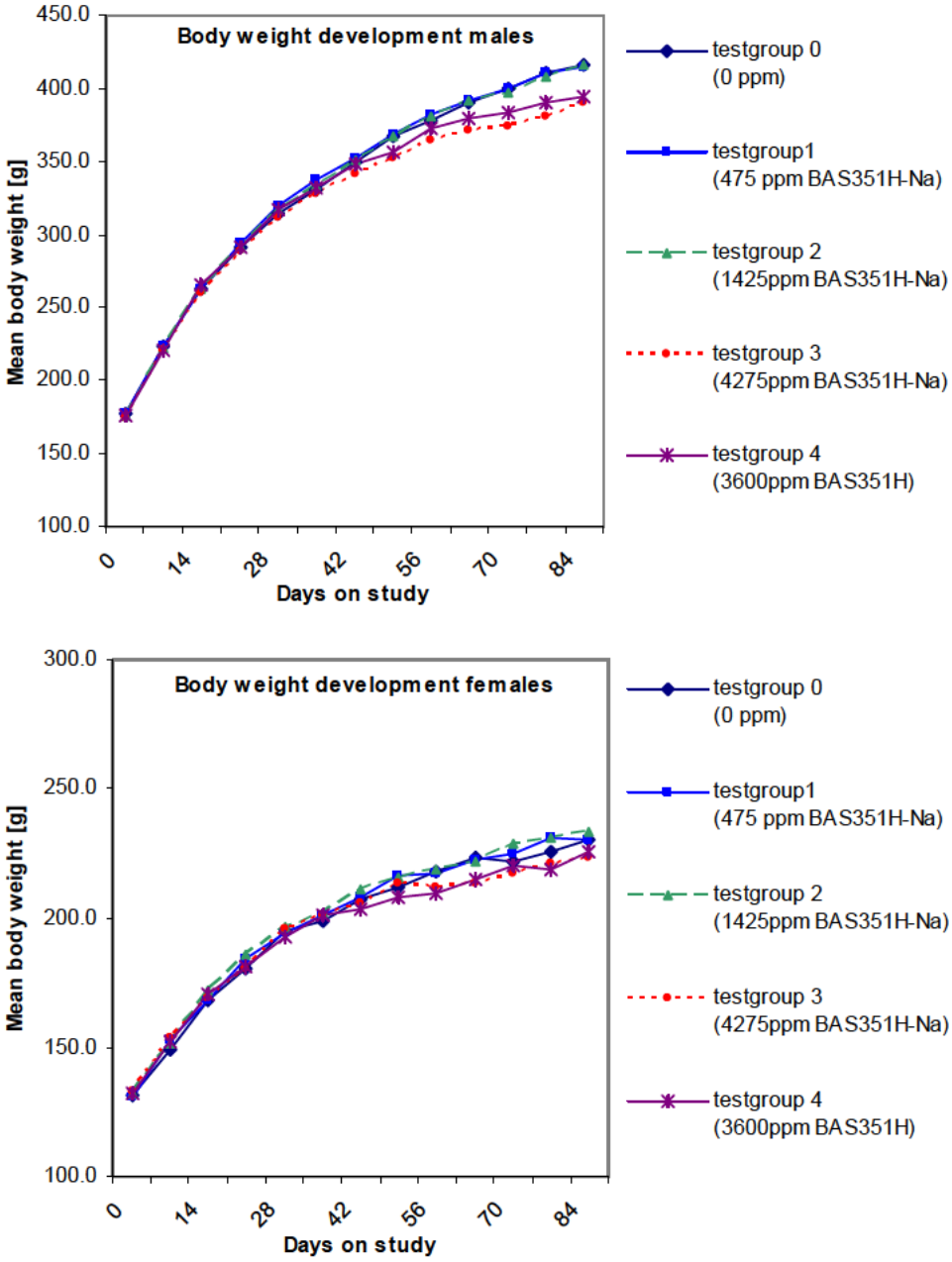


Table 5.3/6 Mean body weight and body weight gain of rats administered Bentazone as sodium-salt or free acid for at least 90 days

Sex	Males					Females				
Test-item	Con-trol	sodium salt			acid	Con-trol	sodium salt			acid
Dose level [ppm]	0 ppm	475 ppm	1425 ppm	4275 ppm	3600 ppm	0 ppm	475 ppm	1425 ppm	4275 ppm	3600 ppm
Mean body weight [g]										
- day 0	177	177.8	175.3	175	176.6	131.3	130.9	132.5	132.8	131.9
- day 91	414.9	414.2	413.7	387.7	396.2	224.9	224.2	226.0	216.6	215.4
□% (compared to control)[#]		-0.2	-0.3	-6.6	-4.5		-0.3	0.5	-3.7	-4.2
Overall body weight gain [g]	237.9	236.4	238.4	212.7	219.6	93.6	93.3	93.5	83.8	83.5
□% (compared to control)[#]		-0.6	0.2	-10.6	-7.7		-0.3	-0.1	-10.5	-10.8

[#] Values may not calculate exactly due to rounding of mean values

These results demonstrate similar body weight effects under Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na) and Reg. No. 51929 (Bentazone-acid, BAS 351 H) treatment in Wistar rats at equimolar dose levels after dietary administration over a period of 3 months.

The overall picture, including the oral 90 day study in Wistar rats performed in 1986 (see Monograph, Vol.3, Annex B-5, September 1996, chapter 5.3.2.2, BASF DocID 1987/0173) with 0, 400, 1200 and 3600 ppm Bentazone (acid-form, batch N 187/ purity 97.8%) and the 90 day oral rat neurotoxicity study with 0, 300, 1000, 3500 ppm Bentazone (BAS 351 H, acid form, batch N 187, purity 96,9%) performed in 2004 (see chapter 5.7.4, BASF DocID 2004/1013171 and 2004/1025741) reveal that Bentazone does not induce significant, reproducible body weight reductions in male of female rats after dietary administration of up to 3600 ppm in its form as free acid, which is equimolar to 4275 ppm Bentazone-sodium. However, there seems to be a slight trend to reduced body weights in both sexes towards the end of the administration period.

E. FOOD CONSUMPTION AND COMPOUND INTAKE

During numerous time intervals, food consumption differed from controls, more increased than decreased values in all treatment groups. Due to a wide variance and the inconsistency of the deviations during the course of the study, irrespective of the dose group, changes are considered to be unrelated to treatment.

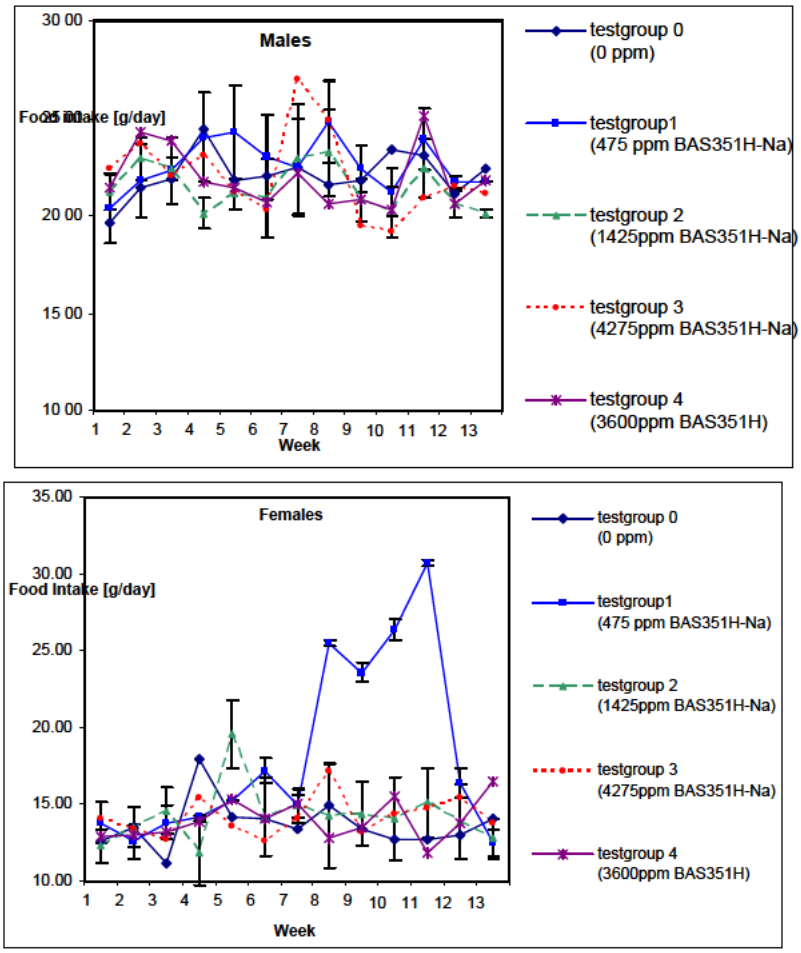
The latter is also evident from the cumulative food intake throughout the entire study (see Table 5.3/7) as calculated by the author of this summary based on the individual mean daily food consumption values, which range between 97.1 and 104.4% of control. The extraordinary high food consumption of the 475 ppm females with 133.3 % of control is, based on the inconsistency in regard to a dose-response relationship and the time course, considered to be incidental and to represent biological variability rather than an effect of treatment.

Table 5.3/7 Cumulative food consumption of rats administered Bentazone as sodium-salt or free acid for at least 90 days

	Males					Females				
Substance	Control	sodium salt			acid	Control	sodium salt			acid
Dose level [ppm]	0	475	1425	4275	3600	0	475	1425	4275	3600
Cumulative food consumption [g/animal]										
day 0 to 91 of treatment [#]	287.0	294.0	278.8	286.8	284.7	177.7	236.8	185.5	184.6	181.3
□% (compared to control) [#]		102.4	97.1	99.9	99.2		133.3	104.4	103.9	102.0

[#] Values were calculated by the author of this summary based on mean individual daily food consumption. Values may not calculate exactly due to rounding of mean values.

Figure 5.3/2 Food consumption of rats administered Bentazone as sodium salt or as free acid for at least 90 days compared to control



The overall picture, including the oral 90 day study in rats (see Monograph, Vol.3, Annex B-5, September 1996, chapter 5.3.2.2 BASF DocID 1987/0173) and the 90 day oral rat neurotoxicity study (see chapter 5.7.4, BASF DocID 2004/1013171 and 2004/1025741) both with Bentazone (BAS 351 H, acid form, batch N 187) reveals that Bentazone does not induce changes in food consumption after dietary administration of its free acid form up to 3600 ppm or after dietary administration of its sodium salt up to equimolar dose levels of 4275 ppm.

The **mean daily test substance intake** of Reg.No. 55691 (Bentazone-sodium) in this study was calculated to have been 31, 91 and 290 mg/kg bw/day in males and 42, 98 and 304 mg/kg bw/day in females at dietary dose levels of 475, 1425 and 4275 ppm. The mean daily test substance intake of Reg.No. 51929 (Bentazone-acid) at 3600 ppm was calculated to be 238 mg/kg bw/day in males and 252 mg/kg bw/day in females (see Table 5.3/8).

The dose levels provide an equimolar substance intake in test group 3 and 4 with 244 versus 238 mg/kg bw/day substance intake in males and 256 versus 252 mg/kg bw/day substance intake in females if calculated as Bentazone-acid. In addition, the test groups 1 and 2, with substance intake of 26 and 76 mg/kg bw/day in males and 35 and 82 mg/kg bw/day in females if calculated as bentazone-acid, are comparable to the 400 ppm and 1200 ppm dose groups of the former 90 day study in rats (see Monograph, Vol.3, Annex B-5, September 1996, chapter 5.3.2.2 BASF DocID 1987/0173), in which a substance intake of 25 and 78 mg/kg bw/day in males and 29 and 86 mg/kg bw/day in females was reached with BAS 351 H (free acid).

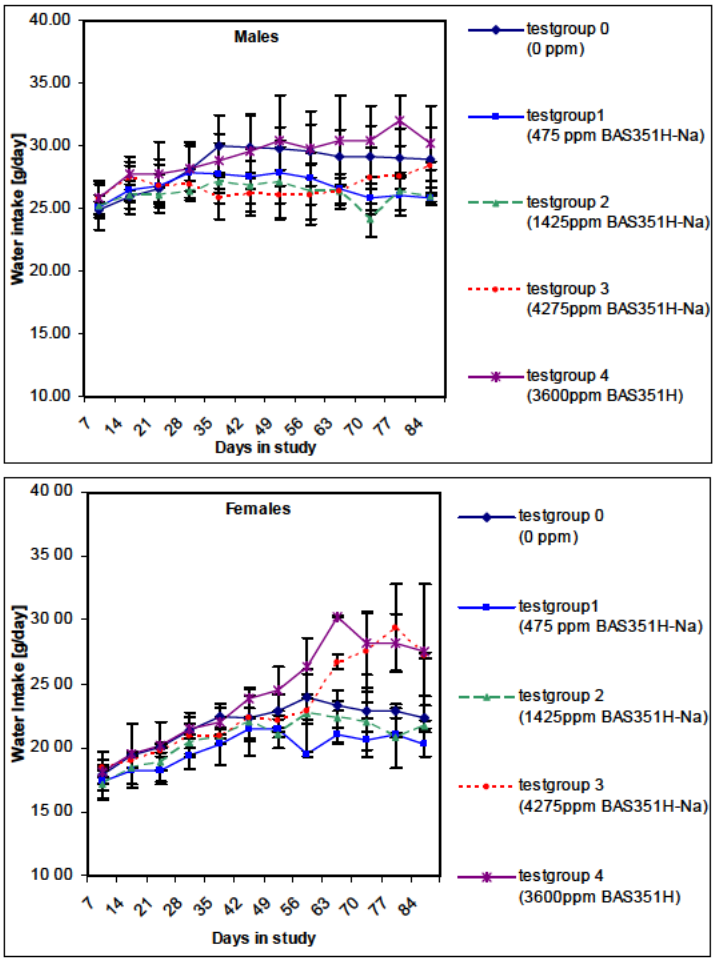
Table 5.3/8 Mean compound intake over the duration of the study

Test group	Concentration in the diet (ppm)	Mean daily test substance intake (mg/kg bw/day)		Mean daily test substance intake calculated equimolar (mg/kg bw/day)	
		Males	Females	Males	Females
1	475 ppm Bentazone-sodium	31	42	26	35
2	1425 ppm Bentazone-sodium	91	98	76	82
3	4275 ppm Bentazone-sodium	290	304	244	256
4	3600 ppm Bentazone-acid	238	252	238	252

F. WATER CONSUMPTION

Due to group housing, only two values of water consumption were obtained per group. Thus, no statistical evaluation was possible. However, considering a deviation greater than 20% to control as relevant, water consumption was increased in females of group 3 (4275 ppm; Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na)) from day 66 onwards and in group 4 (3600 ppm; Reg. No. 51929 (Bentazone-acid, BAS 351 H)) from day 59 onwards. In males no deviation greater than 20% was observed (see Figure 5.3/3).

Figure 5.3/3 Mean daily water consumption in rats administered Bentazone as sodium salt or free acid for at least 90 days (The last week value for water consumption is not presented since the animals were fasted during urinalysis at day 88 (m) and day 89 (f) as well as on day 91)



A comparison with other studies performed with Bentazone reveal:
Increased water consumption was seen occasionally in males and consistently in females in the 2 year carcinogenicity study in Fisher F344 rats (see Monograph, Vol.3, Annex B-5, September 1996, chapter 5.5.2 BASF DocID 1985/433 and amendments) from week 29 onwards at 800 ppm (47/55 mg/kg bw for males/females, respectively). In the 4000 ppm group both males and females showed increased water consumption from week 6 / 17 onwards (233/274 mg/kg bw) for males/females, respectively. Increased water consumption was also seen in the prenatal developmental toxicity study in SD rats with dietary administration from gestation day 0-21 in the 4000 ppm dose group (324 mg/kg bw/day) (see Monograph, Vol.3, Annex B-5, September 1996, chapter 5.6.2.3 BASF DocID 1984/066). No water consumption increase was seen in 28-day studies, confirming that increased water consumption is a delayed phenomenon at least in non pregnant rats.

Under consideration of the entire dataset, a consistent increase in water consumption was observed at least in females. Therefore, this effect is considered to be treatment-related. Additionally, this effect was induced to a similar extent by Reg. No. 88691 (Bentazone-sodium) and Reg. No. 51929 (Bentazone-acid), demonstrating the equivalence of both test-items.

G. BLOOD ANALYSIS

1. Hematological findings

No treatment-related, adverse changes among red and white blood cell parameters were measured.
At the end of the study, in males of test groups 3 (4275 ppm; Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na)) and 4 (3600 ppm; Reg. No. 51929 (Bentazone-acid, BAS 351 H)) activated partial thromboplastin time (PTT) and prothrombin time (QT) were prolonged (see Table 5.3/9). This effect is consistently found in male rats after treatment with bentazone, and, as demonstrated in this study, induced by the acid and sodium form of Bentazone to the same extent.
The prolonged activated partial thromboplastin time and prothrombin time are caused by lower concentrations of coagulation factors. The cause of this lower coagulation factor levels – decreased synthesis or increased consumption in the circulation – is unclear.
In contrast to that, in females of test groups 2 and 3 (1425 ppm and 4275 ppm; Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na)) prothrombin time (QT) was reduced. Because this change was marginal (alteration of means < 7%), it was neither seen in females of the related test group 4 (3600 ppm; Reg. No. 51929 (Bentazone-acid, BAS 351 H)) nor the activated partial thromboplastin time (PTT) was altered in females of the respective groups, this change was regarded incidental and not treatment-related. A reduced thromboplastin time was also described for females in the 90 day rat study performed in 1986 in Wistar rats (see Monograph, Vol.3, Annex B-5, September 1996, chapter 5.3.2.2 BASF DocID 1987/0173) with Bentazone administered in its acid form. Also here the effect was marginal and not dose-related. Based on the fact that bentazone in its acid form did not induce the effects in the current study strengthen the conclusion that this effect is incidental and represent biological variability rather than an effect of treatment.

Table 5.3/9 Selected hematology findings in rats administered Bentazone as sodium salt or free acid for at least 90 days (group means)

Sex		Males					Females				
Test-item		Contr.	BAS 351 H-Na			BAS 351H	Contr.	BAS 351 H-Na			BAS 351H
Dose [ppm]		0	475	1425	4275	3600	0	475	1425	4275	3600
QT [sec]	Mean	18.0	17.6	18.1	**20.2***	**19.8***	17.5	16.9	**16.3***	**16.3***	17.2
	□% #		98%	101%	112%	110%		97%	93%	93%	98%
PTT [sec]	Mean	19.9	19.8	19.9	**23.0****	**22.4****	20.7	20.1	19.5	20.7	20.9
	□% #		99%	100%	116%	113%		97%	94%	100%	101%

* p ≤ 0.05; ** p ≤ 0.01 Kruskal-Wallis + Wilcoxon test (two-sided)
# Δ% procentual deviation from control

In females of test group 4 (3600 ppm; Reg. No. 51929 (Bentazone-acid, BAS 351 H)) lower mean corpuscular hemoglobin content (MCH) and mean corpuscular hemoglobin concentrations (MCHC) compared to controls were calculated. Because no measured red blood cell parameter (hemoglobin, red blood cell counts, hematocrit), but only calculated parameters were changed, these alterations were regarded as incidental and not treatment-related.

Table 5.3/10 Selected hematology findings in rats administered Bentazone as sodium salt or free acid for at least 90 days (group means)

Sex		Males					Females				
Substance		Contr.	BAS 351 H-Na			BAS 351H	Contr.	BAS 351 H-Na			BAS 351H
Dose [ppm]		0	475	1425	4275	3600	0	475	1425	4275	3600
MCH [fmol]	Mean	1.02	1.01	1.04	1.01	1.04	1.11	1.08	1.09	1.09	**1.08***
	□% #		99%	102%	99%	102%		97%	98%	98%	97%
MCHC[mmol/L]	Mean	20.22	20.19	20.39	20.13	20.31	20.43	20.35	20.14	20.23	**20.20***
	□% #		100%	101%	100%	100%		100%	99%	99%	99%

* p □ 0.05; ** p □ 0.01 *** p □ 0.001 Kruskall-Wallis (two-sided)
□% # procentual deviation from control

2. Clinical chemistry findings

No treatment-related, adverse changes among enzymes (ALT, AST, ALP and GGT) were measured. For substrates and electrolytes & minerals some statistically significant clinical chemistry findings were observed in females (see Table 5.3/11).

Table 5.3/11 Selected clinical chemistry findings in rats administered Bentazone as sodium salt or free acid for at least 90 days (group means)

Sex		Males					Females				
Substance		Contr.	BAS 351 H-Na			BAS 351 H	Contr.	BAS 351 H-Na			BAS 351H
Dose [ppm]		0	475	1425	4275	3600	0	475	1425	4275	3600
GLOB [g/l]	Mean	27.4	26.16	25.86	25.38	25.22	25.42	25.58	26.12	**24.62***	**24.38***
	□% #		95%	94%	93%	92%		101%	103%	97%	96%
CHOL [mmol/L]	Mean	1.77	1.88	1.8	1.95	1.91	1.51	1.67	1.78	1.73	**1.98***
	□% #		106%	102%	110%	108%		111%	118%	115%	131%
TRIG [mmol/L]	Mean	0.72	0.82	0.88	0.85	0.93	0.37	0.44	0.38	0.49	**0.56***
	□% #		114%	122%	118%	129%		119%	103%	132%	151%
K+ [mmol/L]	Mean	4.64	4.62	4.64	4.53	4.47	3.84	3.97	4.03	4.05	**4.33****
	□% #		100%	100%	98%	96%		103%	105%	105%	113%

* p □ 0.05; ** p □0.01;Kruskal-Wallis or Kruskal-Wallis-Wilcoxon test (two-sided)
# □% was calculated by the author of this summary based on the mean values given in the report compared to the corresponding control values.

At the end of the study, in females of test group 4 (3600 ppm; Reg. No. 51929 (Bentazone-acid, BAS 351 H)) globulin levels were decreased, whereas cholesterol, triglyceride and potassium mean concentrations were increased. In females of test group 3 (4275 ppm; Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na)) globulin levels were also decreased.
The former 90 day study in Wistar rats performed with Bentazone acid up to 3600 ppm (see Monograph, Vol.3, Annex B-5, September 1996, chapter 5.3.2.2 BASF DocID 1987/0173) revealed increased total cholesterol levels for high dose females but no effect on globulin or triglycerid levels. Effects in males were restricted to increased albumin fraction and albumin to globulin ratio. Potassium levels were not affected but sodium levels were increased.

Under consideration of the entire dataset, a consistent effect is seen only for the increase of cholesterol in females with Reg.No. 51929 (Bentazone-acid), but not with the related Bentazone-sodium salt. This increase in cholesterol levels is indicative for some effects on liver; however, examinations revealed no histopathological changes at any dose level. Therefore this effect is considered as adaptive effect and not regarded as adverse.

3. Urinalysis

At the end of the study, in males of test group 4 (3600 ppm; Reg. No. 51929 (Bentazone-acid, BAS 351 H)) urinary specific gravity was lower compared to controls. In these individuals lower specific gravity was combined with higher urine volume. The same trend was seen with the medians in females of test group 4 regarding urinary specific gravity and urine volume although the alterations were not statistically significant.
This trend for higher urinary output and decreased specific gravity is considered to be treatment-related as it is also found in the former 90 day study for both sexes at 3600 ppm (see Monograph, Vol.3, Annex B-5, September 1996, chapter 5.3.2.2 BASF DocID 1987/0173) and in the carcinogenicity study in the rat after 6 months in both sexes at 4000 ppm (see Monograph, Vol.3, Annex B-5, September 1996, chapter 5.5.2 BASF DocID 1985/433 and amendments).

Table 5.3/12 Selected clinical urine parameters in rats administered Bentazone as sodium salt or free acid for at least 90 days (group means and in part medians)

Sex		Males					Females				
Substance		Contr.	BAS 351 H-Na			BAS 351 H	Contr.	BAS 351 H-Na			BAS 351H
Dose [ppm]		0	475	1425	4275	3600	0	475	1425	4275	3600
TRANS EC_C	Mean	1	1	**2***	1	1	1	1	1	1	1
Volume [ml]	Mean	6	5.2	5.8	6.2	6.5	4.5	2.7	3.8	4.1	4.2
	□% #		87%	97%	103%	108%		60%	84%	91%	93%
	Median	5.8	4.9	5.0	5.9	6.3	4	2.3	4.1	4.2	4.3
	□% #		0.84	0.86	1.02	1.09		0.58	1.03	1.05	1.08
SP.GR._C [g/L]	Mean	1.038	1.039	1.035	1.036	**1.033***	1.037	1.055	1.038	1.035	1.038
	□% #		100.1%	99.7%	99.8%	99.5%		101.7%	100.1%	99.8%	100.1%
	Median	1.037	1.038	1.035	1.036	1.034	1.037	1.053	1.035	1.033	1.032
	□% #		100.1%	99.8%	99.9%	99.7%		101.5%	99.8%	99.6%	99.5%
pH_M	Mean	6.16	6.31	6.29	6.25	6.34	5.68	5.70	5.69	**5.38****	5.54
	□% #		102.4%	102.1%	101.5%	102.9%		100.4%	100.2%	94.7%	97.5%

* p □ 0.05; ** p □0.01;Kruskal-Wallis + Wilcoxon test (two-sided) except for Trans EC_C (one-sided) Wilcoxon
# □% was calculated by the author of this summary based on the mean values given in the report compared to the corresponding control values.

Other findings were, based on the lack of dose-dependency and in combination with the lack of further affected biomarkers, regarded as incidental and not treatment related. These were:

- higher counts of transitional epithelial cells in the urine sediment in males of test group 2 (1425 ppm; Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na))
- higher urinary specific gravity compared to controls in females of test group 1 (475 ppm; Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na))

Under consideration of the entire dataset, a trend for lower urine specific gravity and higher urine volume in both sexes of the high dose group with Reg.No. 51929 (Bentazone-acid), but not with the related Bentazone-sodium salt, was seen.

H. NECROPSY

1. Organ weight

Several organs showed statistically significant changes in absolute or relative organ weights after Bentazone administration for at least 90 days (see Table 5.3/13).

Table 5.3/13 Selected mean absolute and relative organ weights of rats administered Bentazone as sodium salt or free acid for at least 90 days

Sex		Males				Females			
Organ weight	Dose [ppm]	Absolute weight	□% #	Relative weight [% of b.w.]	□% #	Absolute weight	□% #	Relative weight [% of b.w.]	□% #
Terminal weight [g]	0	391.16				211.04			
BAS 351 H-Na	475	392.40	100%			211.59	100%		
	1425	392.19	100%			215.32	102%		
	4275	365.17	93%			201.99	96%		
BAS 351 H	3600	374.68	96%			201.87	96%		
Liver [g]	0	8.734		2.224%		4.854		2.302%	
BAS 351 H-Na	475	8.706	100%	2.231%	100%	5.122	106%	2.419%	105%
	1425	8.128	93%	2.072%	93%	5.074	105%	2.359%	102%
	4275	8.038	92%	2.202%	99%	5.014	103%	**2.481%****	**108%**
BAS 351 H	3600	8.351	96%	2.232%	100%	4.977	103%	**2.467%****	**107%**
Kidneys [g]	0	2.380		0.609%		1.346		0.638%	
BAS 351 H-Na	475	2.322	98%	0.595%	98%	1.367	102%	0.646%	101%
	1425	2.268	95%	0.579%	95%	1.422	106%	0.660%	104%
	4275	2.416	102%	**0.662%***	**109%**	**1.622****	**121%**	**0.803%****	**126%**
BAS 351 H	3600	2.451	103%	0.665%	108%	**1.587****	**118%**	**0.786%****	**123%**
Heart [g]	0	1.060		0.271%		0.674		0.320%	
BAS 351 H-Na	475	1.065	100%	0.272%	100%	0.695	103%	0.328%	103%
	1425	1.043	98%	0.267%	98%	0.695	103%	0.323%	101%
	4275	0.966	91%	0.265%	98%	0.686	102%	0.340%	106%
BAS 351 H	3600	1.003	95%	0.268%	99%	0.690	102%	**0.342%***	**107%**
Adrenals [mg]	0	61.60		0.016%		74.3		0.035	
BAS 351 H-Na	475	57.00	93%	0.015%	92%	73.8	99%	0.035	99%
	1425	57.80	94%	0.015%	94%	76.2	103%	0.036	101%
	4275	59.50	97%	0.016%	103%	69.4	93%	0.035	98%
BAS 351 H	3600	60.20	98%	0.016%	102%	**67.0***	**90%**	0.033	94%
Thymus [mg]	0	0.312		0.080%		0.259		0.123%	
BAS 351 H-Na	475	0.340	109%	0.088%	109%	0.269	104%	0.128%	104%
	1425	0.312	100%	0.079%	99%	0.271	105%	0.127%	103%
	4275	0.305	98%	0.083%	104%	0.245	95%	0.121%	98%
BAS 351 H	3600	0.301	96%	0.080%	100%	**0.295***	**114%**	**0.146%***	**118%**
Brain [g]	0	2.057		0.529%		1.934		0.916%	
BAS 351 H-Na	475	2.057	100%	0.529%	100%	1.894	98%	0.899%	98%
	1425	2.060	100%	0.526%	100%	1.925	100%	0.899%	98%
	4275	2.085	101%	**0.573%***	**108%**	1.926	100%	0.956%	104%
BAS 351 H	3600	2.079	101%	0.557%	105%	1.913	99%	0.950%	103%

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)
# Values may not calculate exactly due to rounding of values given in the report

The main treatment-related effect was seen in the kidneys of females in test group 3 (4275 ppm Bentazone-sodium) and 4 (3600 ppm Bentazone-acid) with increased absolute and relative kidney weights, although a morphologic correlate was not detected. In the respective high dose males, also without histopathological correlate, only the relative kidney weights were increased, statistically significant only in the high dose Bentazone-sodium group. Kidney weight increase was repeatedly documented in former studies after Bentazone treatment. This effect might be partly a consequence of a higher water throughput and therefore of minor toxicological relevance.
In females increased relative liver weights were found in test group 3 (4275 ppm Bentazone-sodium) and 4 (3600 ppm Bentazone-acid), but a treatment–related effect is questionable and rather unlikely as a clear dose response relationship and a histological correlate was missing.

The increase of relative brain weights in males and relative heart weights in females is considered secondary to the reduced terminal body weight and thus not of toxicological relevance.
In absence of any corroborative histopathological change and without a clear dose-response the increase in thymus (absolute and relative weights) in bentazone-acid treated females is regarded as unlikely to be treatment-related. This conclusion is supported by the former 90 day study in rats (no changes in thymus weight) and the 6 month sacrifice data in the 2 year carcinogenicity study showing reduced thymus weight (see Monograph, Vol.3, Annex B-5, September 1996, chapter 5.3.2.2 BASF DocID 1987/0173 and chapter 5.5.2 BASF DocID 1985/433 and amendments).
In adrenal (absolute weights in females) a treatment–related effect is unlikely as a clear dose response relationship and a histological correlate was missing.

No other statistically significant changes of absolute or relative organ weights were observed.

2. Gross lesions and histopathology

All macroscopic findings occurred either individually or were biologically equally distributed over control and treatment groups. These consisted of erosion/ulcer of the glandular stomach in each one male of the control group, the low and high dose Bentazone-sodium treated group and the Bentazone-acid treated group. Liver deformation and deposition were seen in one male of the Bentazone-acid group and one liver focus in a low dose Bentazone-sodium treated male. In females, gross lesions consisted of each one female with renal pelvic dilation in the mid-dose Bentazone-sodium group, enlarged liver in the Bentazone-acid group and hepatic necrosis in the low dose Bentazone-sodium group. Furthermore, one female of the mid-dose Bentazone-sodium group showed a dilated uterus. These findings are considered to be incidental or spontaneous in origin and without any relation to treatment (see Table 5.3/14).

Table 5.3/14 Incidence of selected macro- and histopathological lesions in rats administered Bentazone as sodium salt or acid for at least 90 days

Sex	Males					Females				
Substance	Contr.	BAS 351 H-Na			BAS 351 H	Contr.	BAS 351 H-Na			BAS 351 H
Dose [ppm]	0	475	1425	4275	3600	0	475	1425	4275	3600
Animals in group	10	10	10	10	10	10	10	10	10	10
Macropathology										
No abnormalities	9	8	10	9	9	10	9	8	10	9
Glandular stomach										
-Erosion/ulcer	1	1		1	1					
Kidney										
-Pelvic dilation								1		
Liver										
-Deformation					1					
-Deposition					1					
-Enlarged										1
-Focus		1								
-Necrosis							1			
Uterus										
-Dilation								1		
Histopathology										
Adrenal cortex	10			10	10	10			10	10
-Vacuolation, increased				2	3					
-Hypertrophy, multifocal						1				
-Accessory cortical tissue	3			2	4				2	2
Glandular stomach	10	1		10	10	10			10	10
-Inflammation, (multi)focal				1					1	1
-Erosion/ulcer	1	1		1	1					
-Metaplasia, basal cell									1	2
-Arteritis,(multi)focal									1	
Kidney	10	10	10	10	10	10		1	10	10
-Mineralization, medulla				1		9		1	9	8
-Nephropathy, chronic[$]	5	6	**8**	**10**	**9**	4			4	1
	[1.2]	*[1.0]*	*[1.25]*	*[1.7]*	*[1.4]*					
-Dilation, renal pelvis								1		
Liver	10	1		10	10	10	1		10	10
-Infiltration, lymphoid	7			7	8	6			6	2
-Torsion of lobe		1					1			
-Fibrosis, (multi)focal	1									
-Fibrosis, perivascular					1					
-Vacuolation, hpatocell.										1
-Necrosis, (multi)focal						1				
Ovaries						10			10	10
-Sertoli cell tubule, focal										1
Pancreas	10			10	10	10			10	10
-Atrophy, acinar cells	1			3	2	2				
Pituitary gland	10			10	10	10			10	10
-Cyst, pars intermedia				1						
-Cyst, pars intermedia									1	
Skin	10			10	10	10			10	10
- Inflammation, (multi)focal									1	
Spleen	10			10	10	10			10	10
-Hematopoiesis, extramed.									1	
Testes	10			10	10					
- Debris in sem. Tubules, (m)f	3			1						
- Degeneration, tubular, (m)f	1			2	1					
Thymus	10			10	10	10			10	10
-Starry sky cells, increased	1			2	2	4			5	5
-Hyperplasia, tub/cords,(m)f	3			4	5	5			6	8
Uterus						10		1	10	10

Table 5.3/14 Incidence of selected macro- and histopathological lesions in rats administered Bentazone as sodium salt or acid for at least 90 days

Sex	Males					Females				
Substance	Contr.	BAS 351 H-Na			BAS 351 H	Contr.	BAS 351 H-Na			BAS 351 H
Dose [ppm]	0	475	1425	4275	3600	0	475	1425	4275	3600
Animals in group	10	10	10	10	10	10	10	10	10	10
- Dilation of horn(s)								1		

$ [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence

A treatment-related increase in incidence of minimal to slight chronic nephropathy was noted in male animals of test group 2 and 3 (1425 and 4275 ppm; Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na)) and 4 (3600 ppm; Reg. No. 51929 (Bentazone-acid, BAS 351 H)). As the severity was not increased over the grading already found in control animals (up to slight severity) this finding is per se not considered to be an adverse finding but a substance-induced exacerbation of a general occurring kidney lesion.

All other histopathological findings were either single observations, were equally distributed between control and treated groups or displayed no dose-response relationship. Therefore, these findings were considered to be incidental or spontaneous in origin and without any relation to treatment.

The findings of the current study are well comparable with the former 90 day dietary study in rats (see Monograph, Vol.3, Annex B-5, September 1996, chapter 5.3.2.2 BASF DocID 1987/0173) in which no treatment-related gross or microscopic pathology findings occurred.

Taken together, regarding pathology, findings noted with Reg.No. 51929 (Bentazone-acid, BAS 351 H) and Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) are well comparable and confirm the data generated in the former 90 day study in rats with Bentazone in its free acid form (see Monograph, Vol.3, Annex B-5, September 1996, chapter 5.3.2.2 BASF DocID 1987/0173).

III. CONCLUSIONS

Dietary administration of Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na) to Wistar rats at dose levels of 0, 475, 1425 ppm and 4275 ppm over a period of 90 days resulted in prolonged activated partial thromboplastin time (PTT) and prothrombin time (QT) in males and decreased globulin values in females at the highest dose. In addition kidney (absolute and relative) weights and relative liver weights were increased in females without histopathological correlate. This organ weight increase was considered to be caused by increased water consumption towards the end of the administration period. There was also a trend to reduced body weights in both sexes towards the end of the study.
For comparison with the high dose Bentazone-sodium group an equimolar dosed group of Reg. No. 51929 (Bentazone-acid, BAS 351 H) which is 3600 ppm was also administered over a period of 3 months. This group showed similar results than the 4275 ppm Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na) group and thereby demonstrated the equivalence of both substances.
Furthermore, the findings were well comparable with the findings of the former subchronic study (see Monograph, Vol.3, Annex B-5, September 1996, chapter 5.3.2.2 BASF DocID 1987/0173).

In conclusion, the comparison of Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na) and Reg. No. 51929 (Bentazone-acid, BAS 351 H) in Wistar rats over a period of 3 months oral administration revealed similar signs of toxicity for both compounds at the high equimolar dose tested.

No signs of toxicity were observed in male and female Wistar rats treated with Reg. No. 88691 (Bentazone-sodium, BAS 351 H-Na) up to a dose level of 1425 ppm (91 mg/kg bw/d in males and 98 mg/kg bw/d in females) being the no observed adverse effect level (NOAEL).

5.3.3 Oral 90-day toxicity (dog)

No new studies were performed. All information available is presented in the original Annex II Dossier (1995) and has been evaluated by European authorities and Germany as Rapporteur member state.

5.3.4 Oral 1 year toxicity (dog)

No new studies were performed. All information available is presented in the original Annex II Dossier (1995) and has been evaluated by European authorities and Germany as Rapporteur member state.

5.3.5 28-day inhalation toxicity (rodents)

BAS 351 H is a non-volatile compound and model exposure data show that inhalation is not a significant route of exposure. For operator risk assessment, the tolerable exposure by inhalation is derived from the systemic AOEL. The exposure assessment for operators and bystanders further assumes that 100% of the airborne exposure is bioavailable, which is considered a worst-case approach. Moreover, the available acute inhalation toxicity study with BAS 351 H did not indicate any specific inhalation toxicity. Therefore, the absence of 28-day and 90-day inhalation toxicity studies in rats is considered not to constitute a data gap.

5.3.6 90-day inhalation toxicity (rodents)

BAS 351 H is a non-volatile compound and model exposure data show that inhalation is not a significant route of exposure. For operator risk assessment, the tolerable exposure by inhalation is derived from the systemic AOEL. The exposure assessment for operators and bystanders further assumes that 100% of the airborne exposure is bioavailable, which is considered a worst-case approach. Moreover, the available acute inhalation toxicity study with BAS 351 H did not indicate any specific inhalation toxicity. Therefore, the absence of 28-day and 90-day inhalation toxicity studies in rats is considered not to constitute a data gap.

5.3.7 Percutaneous 21-day toxicity (rodents)

No new studies were performed. All information available is presented in the original Annex II Dossier (1995) and has been evaluated by European authorities and Germany as Rapporteur member state.

5.3.8 Percutaneous 90-day toxicity (rodents)

The results from the available 21-day dermal toxicity study in rabbits have shown that Reg.No. 51 929 has a low order of dermal toxicity and thus has not revealed any cause for concern. Therefore, the absence of a 90-day percutaneous toxicity study in rats is considered not to constitute a data gap.

5.4 Genotoxicity

Studies presented in the original Annex II Dossier (1995): Bentazone as free acid was investigated for mutagenicity in numerous studies using various test systems and 6 different batches. These investigations included point and gene mutation, chromosomal aberration and mitotic recombination as well as DNA damage and repair studies. **Bentazone-sodium** was additionally tested in a bacterial system for its ability to induce point mutations. The studies were conducted more or less in line with the pre-1991 mutagenicity guideline requirements. Most studies are without GLP according to the practice in those days and substance information regarding batch and purity are only partially available. However, the weight of evidence points to an absence of genotoxic potential at least in vivo. Therefore, no further data were considered to be generated on Bentazone-acid. The studies have been evaluated by European authorities and Germany as Rapporteur member state in 1998 (European Commission Peer Review Program, BASF Doc ID 1998/1001178) and are, for the convenience of the reviewer, listed in Table 5.4/1.

Based on these genotoxicity studies, it can be summarized that bentazone does not induce point mutations in bacterial or mammalian test systems in the presence or absence of metabolic activation. The weakly mutagenic effect found in one HPRT point mutation test on CHO cells was not reproducible. Results of cytogenetic studies in vitro were negative with bentazone but it is worth to mention that in the Monograph a positive Chromosome aberration study is listed (see Monograph, Vol.3, Annex B-5, September 1996, chapter 5.4.1.3.6 de Vogel N and Wilmer JWGM, 1986; Report no. V 86.517/260915). Therefore it was agreed that bentazone might possess clastogenic activity in vitro. Nevertheless, all tests for chromosomal aberration in vivo, including germ cells, were negative indicating the lack of a clastogenic potential of bentazone at least in mammals. No evidence of DNA damage was observed in vitro in bacterial and mammalian test systems or in in vivo study. Bentazone-sodium was also tested negative in a bacterial system for its ability to induce point mutations.

Considering the weight of evidence, it was agreed that bentazone has no mutagenic or genotoxic properties in all three endpoints of the genetic damages tested.

Table 5.4/1 Summary of peer-reviewed Genotoxicity studies with Bentazone

Study type	Test system	Substance Dose / concentr. range (batch / purity)	Result a) without b) with S9-mix	Reference (BASF DocID)
Bacterial reverse mutation assay (Ames test)	*S.typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100; *E.coli* strain WP2 hcr	Bentazone 0-10-50-100-500-1,000-µg/plate (batch not given/ 94%)	a) negative b) negative	Shirasu Y et al., 1976 a Doc ID 1976/009
Bacterial reverse mutation assay (Ames test)	*S.typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100; *E.coli* strain WP2 hcr	Bentazone 0-1,000-2,500-5,000-10,000 µg/plate (batch not given/ 94%)	a) negative b) negative	Moriya M, 1984a Doc ID 1984/10285 Addendum to 1976/009
Bacterial reverse mutation assay (Ames test)	*S.typhimurium* strains TA 1537, TA 98, TA 100;	Bentazone (77/357) 0-3.1-10-31-100-310-1,000-2,000 µg/plate (batch not given/ 92.5%)	a) negative b) negative	Oesch F. 1977 a Doc ID 1977/028
Bacterial reverse mutation assay (Ames test)	*S.typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100;	Bentazone (83/3) 0-20-100-500-2,500-5,000 µg/mL (batch not given/ 96.7%)	a) negative b) negative	Engelhardt G., & Gelbke H.-P., 1983a Doc ID 1983/222
Bacterial reverse mutation assay (Ames test & E.coli reverse mutation assay)	*S.typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100; *E.coli* strain WP2 uvrA	Bentazone 0-20-100-500-2,500-5,000 µg/mL (N169 / 92.6%%)	a) negative b) negative	Engelhardt G., & Gelbke H.-P., 1985a Doc ID 1985/108
Bacterial reverse mutation assay (Ames test)	*S.typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100;	Bentazone-sodium (pure and technical grade) 0-500-1,000-2,500- 5,000-7,500-10,000 µg/mL (84/298/ not given; 84/299/ 550g/l)	a) negative b) negative	Engelhardt G., & Gelbke H.-P., 1985b Doc ID 1985/081
Published literature: Bacterial reverse mutation assay (Ames test)	*S.typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100; *E.coli* strain WP2 hcr	Bentazone Up to 5,000 µg/mL (batch&purity not given)	a) negative	Moriya M et al., 1983a Doc ID 1983/10177
Published literature: Bacterial reverse mutation assay (Ames test)	*S.typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100; *E.coli* strain WP2 hcr	Bentazone (not given / 99.9%)	a) negative b) negative	Jeang C.-L., Li G.-C., 1978 a Doc ID 1978/10236 Jeang C.-L., Li G.-C., 1978 a Doc ID 1980/10294
Published literature: Bacterial reverse mutation assay (Ames test & E.coli reverse mutation assay)	*S.typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100; *E.coli* strain WP2 hcr	Bentazone (batch&purity not given)	a) negative b) negative	Shirasu Y et al., 1982a; Doc ID 1982/10230
Published literature: DNA damage and repair (SOS Chromotest)	*E.coli*	Bentazone (batch&purity not given)	a) negative b) negative	Xu H.H & Schurr K.M., 1990a Doc ID 1990/10692
Published literature: DNA damage and repair (Mitotic gene conversion assay)	*Saccharomyces cerevisiae* strain D4	Bentazone (batch&purity not given)	a) negative	Siebert D.,Lemperle E., 1974a Doc ID 1974/10200

Table 5.4/1 Summary of peer-reviewed Genotoxicity studies with Bentazone

Study type	Test system	Substance Dose / concentr. range (batch / purity)	Result a) without b) with S9-mix	Reference (BASF DocID)
Published literature: DNA damage and repair (Mitotic gene conversion assay)	*Saccharomyces cerevisiae* strain D4	Bentazone (batch&purity not given)	a) negative	Zimmermann F.K. et al., 1984 a Doc ID 1984/10261
In-vitro chromosome aberration assay in mammalian cells	CHO cells	Bentazone a) 0-500-1000-2,000-3,000 µg/ml b) 0-2,000-3,000-4,000-5,000 µg/ml (batch&purity not given)	a) negative b) negative	Taalman R.D.F.M., 1987a Doc ID 1987/0169
In-vitro forward mutation assay in mammalian cells (HPRT test)	CHO cells	Bentazone techn. (84/140) 0-100-464-1,000-2,150-4,640-10,000 µg/ml (N 169/ 93.9%)	a) negative b) negative with rat S9-mix c) weakly positive with mice S9-mix	Jaeckh R., Gelbke H.-P., 1985a Doc ID 1985/396
In-vitro forward mutation assay in mammalian cells (HPRT test)	CHO cells	Bentazone techn. (84/140) 1,250-2,500-5,000-7,500-10,000-12,500-15,000 µg/ml (batch not given/ 93.9%)	a) negative b) negative with rat S9-mix c) negative with mice S9-mix	Boer W.C., den, 1985 a Doc ID 1985/403
In-vitro forward mutation assay in mammalian cells (HPRT test)	CHO cells	Bentazone techn. 0-100-600-1,200-2,500-5,000 µg/ml (N194/ 97.6%)	a) negative b) negative	Muellerschoen H., 1991 a Doc ID 1991/11108
Chromosome analysis in vivo (Micronucleus test)	NMRI Mouse	Bentazone techn. 0-200-400-800 mg/kg bw single application (MS2F22/ 95.6%)	negative	[redacted] 1985c Doc ID 1985/036
Published literature: Chromosome analysis in vivo (Chromosome analysis and Micronucleus test)	Wistar rats	Bentazone techn. 0-27,5-55-110-220-700 mg/kg bw Two oral administrations with 24 h time lag (batch&purity not given)	negative	Postica F. et al., 1982a Doc ID 1982/10236
In-vivo Unscheduled DNA Synthesis (UDS)	B6C3F1 mice	Bentazone techn. (84/140) 40-360 mg/kg bw single application	negative	[redacted] 1982a Doc ID 1985/159
In-vivo mutation assay in germ cells (Dominant lethal test in vivo	Rat (Sprague-Dawley)	Bentazone Dietary concentration: 20-60-180 ppm over 13 weeks (batch&purity not given)	negative	[redacted] 1971a Doc ID 1971/018
In-vivo mutation assay in germ cells (Dominant lethal test in vivo	Mouse (NMRI)	Bentazone techn. Single intraperitoneal application: 195 mg/kg bw (batch&purity not given)	negative	[redacted] 1973 a Doc ID 1973/025

Table 5.4/1 Summary of peer-reviewed Genotoxicity studies with Bentazone

Study type	Test system	Substance Dose / concentr. range (batch / purity)	Result a) without b) with S9-mix	Reference (BASF DocID)
In-vitro Unscheduled DNA Synthesis (UDS)	Mouse Primary hepatocytes (B6C3F1)	Bentazone (84/110) 0.05 up to 1004 µg/ml in WME medium	negative	Cifone M.A., 1985b Doc ID 1985/067

Submission of not yet peer-reviewed studies:
Regarding the higher impurity amounts of the manufactured use product Bentazone-sodium (BAS 351 H-Na, Reg.No. 88691; Batch COD-001417, purity 91.9% ± 1%), the notifier performed a complete battery of genotoxicity test *in-vitro* and *in-vivo*. The studies are summarized in Table 5.4/2.

Exposure of Salmonella typhimurium and Escherichia coli tester strains to Reg.No. 88691 at concentrations up to and including the limit concentration of 5500 µg/plate, which considers the degree of purity of the manufactured in use product, did not produce an increased number of reversions, neither with nor without metabolic activation. An *in-vitro HPRT* assay in CHO cells for the assessment of the gene mutation potential in prokaryotic and eukaryotic systems was negative, with and without S-9 mix, when tested at sufficiently high concentrations of up to 10 mM.
The capability of the test substance for clastogenicity/aneugenicity was assessed using the in vitro chromosome aberration assay in Chinese hamster V79 cells. In this assay Reg.No. 88691 did induce biologically relevant structural chromosome aberrations with and without S-9 mix to a similar degree, reaching statistical significance at the highest concentration of 3000 µg/mL. These observations were made after a 4h treatment period. Reg.No. 88691 did not induce numerical chromosome aberrations, neither with nor without S-9 mix.

The relevance of the observed in vitro mutagenicity was assessed in-vivo using the murine bone marrow micronucleus assay and as follow up, using a different endpoint in a second target tissue, in an Unscheduled DNA Synthesis test in rat hepatocytes. In the bone marrow micronucleus test in NMRI mice the substance was tested up to 1370 and 870 mg/kg bw in males and females (approximately 1250 and 800 mg/kg bw active ingredient), leading to weak signs of toxicity (piloerection from h1-d1). These dose levels were chosen based on severe toxicity observed in males at 1500 mg/kg bw active ingredient and mortality in females at dose levels of 1000 mg/kg bw active ingredient. Although the bioavailability of the test substance in the mouse after oral exposure was not directly assessed in this study, the systemic bioavailability can be deduced by the signs of toxicity and by the plasmakinetic data in general which demonstrated a high bioavailability of the active ingredient after oral administration in all tested species. The results of the micronucleus test did not show any indication of a biologically relevant clastogenic potential, irrespective of dose and the preparation interval (24 and 48 h intervals).
In the second in vivo assay to clarify the relevance of the in vitro mutagenicity, no indications of an induced increase in the DNA repair activity in the hepatocytes of male Wistar rats was measured by autoradiography at any of the tested intervals (3 and 14 h after exposure). The Wistar rats had been treated orally with up to 550 mg Reg.No. 88691/kg bw. The highest dose of 1100 mg Reg.No. 88691/kg bw was not evaluated due to mortalities. Also here, the bioavailability of the test substance in the rat was not directly assessed in the study but can be deduced from the signs of toxicity and in general from the plasmakinetic studies, in which the test substance was detected in the liver after oral administration of bentazone sodium to rats.

In the literature a regulatory not relevant wing spot test of Drosophila melanogaster with Bentazone (purity>95%) is reported using both standard and high-bioactivation fly crosses. In this in vivo screening test Bentazone gave a negative response in the standard cross but a positive result in the high-cytochrome P-450-dependent bioactivation cross. This typ of response is not regarded as evidence for clastogenic activity but indicate in general some genotoxic capacity after bioactivation or degradation in the high-bioactivation Drosophila melanogaster cross.

Furthermore, a QSAR analysis has been performed on the structure of the active ingredient and the diverse impurities. No genotoxicity alert was found (see confidential information in Bentazone AIR2 Dossier Document JM-II 5.8).

In summary the above stated in vivo studies did not confirm the in vitro observation that the test substance induces chromosome aberrations with and without metabolic activation in regulatory studies. Additionally, the structures of the impurities are not linked to any genotoxic alert. Thus, the observed in vitro clastogenic effect of bentazone-sodium is attributed to the chemical structure of the active ingredient but is evaluated as biologically not relevant and the test substance Reg.No. 88691, as well as Reg.No. 15929 (Bentazone-acid), is therefore evaluated as being non mutagenic.

Table 5.4/2 Genotoxicity studies with Bentazone-sodium for evaluation in the AIR II process

Study type	Test system	Dose / concentr. range* (batch / purity)	Result	Reference (BASF DocID)
Bacterial reverse mutation assay (Ames test)	S.typhimurium strains TA 1535, TA 1537, TA 98, TA 100; E.coli strain WP2 uvrA; plate incorporation and pre-incubation assay; with/without S-9 mix	33-100-333-1000-2750-5500 µg/plate (COD-001417 / 91.9%)	negative	AIR2-Dossier II A 5.4.1/1 Woitkowiak, 2011 DocID 2011/1106427
In-vitro chromosome aberration assay in mammalian cells	V79 cells; with/without S-9 mix	187.5-375-750-1500-3000 µg/mL (COD-001417 / 91.9%)	positive for clastogenicity negative for aneugenicity with and without S9	AIR2-Dossier II A 5.4.2/1 Schulz & Landsiedel, 2011 DocID 2011/1106426
In-vitro forward mutation assay in mammalian cells (HPRT test)	CHO cells; with/without S-9 mix	187.5-375-750-1500-3000 µg/mL without S9 187.5-375-750-1500-3000 and 536-1071-2143-3000 µg/mL with S9 (COD-001417 / 91.9%)	negative	AIR2-Dossier II A 5.4.3/1 Schulz & Landsiedel, 2011 Doc ID 2011/1184494
In-vivo micronucleus test	NMRI mouse, ♂ & ♀, single oral (gavage) application; vehicle: Deionized water)	♂: 340-680-1360 mg/kg bw ♁:217.5-435-870 mg/kg bw (COD-001417 / 91.9%)	negative	AIR2-Dossier II A 5.4.4/1 [redacted] 2011 (c) Doc ID 2011/1184970 AIR2-Dossier II A 5.4.4/1 [redacted] 2011 (e) Doc ID 2011/1277971
In-vivo Unscheduled DNA Synthesis (UDS)	Wistar rat, ♂ (single oral gavage); analysis of rat hepatocytes prepared 3 or 14 h after exposure	0-275-550-1100 mg/kg bw (COD-001417 / 91.9%)	negative	AIR2-Dossier II A 5.4.5/1 [redacted] 2011 (d) Doc ID 2011/1192857 AIR2-Dossier II A 5.4.5/2 [redacted] 2011 (f) DocID 2011/1277972
Literature: Wing spot test on somatic mutation and recombination	Drosophila melanogaster, standard and high bioactivation fly crosses,	0.05 mM - 5 mM (not reported/>95%)	negative in standard, positive in high bioactivation fly cross	AIR2-Dossier II A 5.4.7/1 Kaya B. et al. 2003 (a) DocID 2011/1257369

* Dose or concentration range is given for the test substance bentazone-sodium, thereby about 10% higher than the concentration of the active ingredient bentazone-acid

In summary, a classification for mutagenicity is considered to be not justified for Bentazone (BAS 351 H) or the manufactured use product Bentazone-sodium (BAS 351 H-Na) on the basis of the genotoxicity studies in accordance to GHS-classification criteria laid down in the Council Regulation 1272/2008/EC and OECD Globally Harmonized System (GHS, 2009).
This is in addition supported by the QSAR analysis performed on the structure of the active ingredient and the diverse impurities, which found no alert for genotoxicity (see confidential information in Bentazone AIR2 Dossier Document JM-II 5.8).

The endpoints which were fixed in the European Commission Review report for the active substance bentazone (7585/VI/97-final from 30 November 2000) for genotoxicity are therefore considered to be still valid and might be supplemented with regard to Bentazone-sodium.

EU agreed Endpoints for bentazone (EC Review report, 2000)

Genotoxicity	no genotoxic potential

5.4.1 In vitro genotoxicity - Bacterial assay for gene mutation

Report:	II A 5.4.1/1 Woitkowiak C. 2011(a) Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) - Salmonella typhimurium / Escherichia coli - Reverse mutation assay BASF DocID 2011/1106427
Date of report:	30-Jun-2011
Testing facility:	BASF SE; Ludwigshafen/Rhein; Germany Fed.Rep.
Guidelines:	OECD 471; EPA 870.5100; (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/14
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium and E. coli were exposed to Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) (Batch COD-001417; purity 91.9%) using water as a solvent in the presence and absence of metabolic activation in two independent sets of experiments. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.
In the plate incorporation assay as well as in the preincubation test, Reg.No. 88691 was tested up to the limit concentration of 5500 µg/plate. Concentrations of 33, 100, 333, 1000, 2750 and 5500 µg/plate were used with and without metabolic activation. In both the plate incorporation assay and the preincubation assay a weak bacteriotoxic effect was occasionally observed depending on the strain and test conditions at concentrations of 2750 µg/plate and above.
Neither in the first experiment (plate incorporation test) nor in the second experiment (preincubation test) a biologically relevant increase in the number of revertant colonies was noticed in any of the strains tested in presence or absence of metabolic activation. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.
According to the results of the study the test substance Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) is not mutagenic in the Salmonella typhimurium/ Escherichia coli reverse mutation assay under the experimental conditions of the study.
(BASF DocID 2011/1106427)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material	Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na)
Description:	Solid, yellowish
Lot/Batch #:	COD-001417
Purity:	91.9 % (tolerance ±1.0%)
Stability of test compound:	The test substance was guaranteed to be stable until 01. Nov 2013, thus over the study period under the storage conditions. The homogeneity of the test substance was ensured by mixing prior to preparation of test substance formulations. The stability of the test substance at room temperature in water each over a period of 4 hours was verified.
Solvent used:	Water

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)

Vehicle control: The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.

Positive control compounds tested without addition of metabolic activation system:

Strain	*Mutagen*	*Solvent*	*Concentration*
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	*Mutagen*	*Solvent*	*Concentration*
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

3. Activation:

S9 was produced from male Wistar rats. The rats were induced by daily application of 80 mg/kg b.w. phenobarbital i.p. and ß-naphthoflavone p.o. on three consecutive days and were sacrificed 24 hours after the last administration. The livers were washed with 150 mM KCL solution, minced and processed for centrifugation at 9000g. The supernatant (so called S9 fraction) was portioned and stored at -70 to -80°C.

The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	*Concentration*
Sodium phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537

E. coli: WP2 uvrA

Salmonella typhimurium:

The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.

Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay:	Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2750 and 5500 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9).
Pre-incubation assay:	The test article / vehicle / positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2750 and 5500 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9).
Remark:	In this study, due to the purity of the test substance 5500 µg/plate (instead of the standard 5000 µg/plate) was used as top dose in both experiments.
Re-tests:	No re-testing was necessary.

B. TEST PERFORMANCE:

1. Dates of experimental work: 07-Mar-2011 - 01-Apr-2011

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Vogel Bonner agar plates (minimal glucose agar plates). In the experiments with E. coli the Vogel Bonner agar plates were replaced by plates containing a SA1 selective agar according to Green and Muriel.

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S 9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

4. Titer determination:

In order to assess bacteriotoxic effects the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37 °C the number of bacterial colonies was determined.

5. Statistics:

No special statistical tests were performed.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9-mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions.

The stability of the test substance at room temperature in the vehicle (water) over a period of 4 hours was verified analytically. The data are presented in below:

Sampling date	Vehicle	Time	Nominal concentration	Concentration found		Percent of nominal
			% [v/v]	Amount [%] [v/v]	Average[%][v/v]	[%]
15.04.2011	in H_2O	0h	1.003	1.002	1.000	99.7
				0.998		
		4h	1.006	1.005	1.005	99.9
				1.005		

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

Weak bacteriotoxic effects (slight decrease in the number of his^+ or trp^+ revertants, slight reduction in the titer) were observed in the standard plate test depending on the strain and test conditions from about 2 750 µg/plate onward.

In the preincubation assay bacteriotoxicity (decrease in the number of his^+ or trp^+ revertants, reduction in the titer) was observed depending on the strain and test conditions from about 2 750 µg/plate onward.

C. SOLUBILITY

No test substance precipitation was found with and without S9 mix.

D. MUTATION ASSAYS

Neither in the original nor in the confirmatory experiment with and without metabolic activation a biologically relevant increase in number of revertants was observed in any strain tested (see Table 5.4/3).
The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system.

Table 5.4/3 Bacterial gene mutation assay with Reg.No. 88691 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Experiment 1 (original): Plate incorporation assay										
Metabol. activation	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Neg. control (water)	24	33	100	111	19	19	8	8	35	43
Reg.No. 88691										
33 µg/plate	26	32	100	105	21	17	10	8	31	45
100 µg/plate	29	27	102	111	20	17	7	9	31	33
333 µg/plate	26	35	105	107	17	16	6	10	33	35
1000 µg/plate	26	26	94	97	20	15	8	8	31	32
2750 µg/plate	23	17	78	91	11	13	7	6	32	31
5500 µg/plate	16	14	60	49	11	10	3	3	22	27
Pos. control[§]	530	826	730	942	953	188	325	187	672	212
Experiment 2 (confirmatory): Preincubation assay										
Metabol. activation	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Neg.control (water)	25	30	102	106	17	18	8	8	32	39
Reg.No. 88691										
33 µg/plate	28	29	114	125	16	16	6	7	42	42
100 µg/plate	27	32	105	103	18	19	8	8	34	38
333 µg/plate	25	26	105	122	19	15	8	7	29	47
1000 µg/plate	28	33	116	106	18	19	7	8	43	40
2750 µg/plate	20	19	95	91	16	13	5	6	27	27
5500 µg/plate	13	12	68	40	9	11	3	1	17	21
Pos. control[§]	539	565	1012	822	645	150	399	126	573	241

[§] = Compound and concentrations see Material and Methods (I.A.2.) above

III. CONCLUSIONS

According to the results of the present study, the test substance Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) is not mutagenic in the Salmonella typhimurium/Escherichia coli reverse mutation assay under the experimental conditions chosen here.

5.4.2 In vitro genotoxicity - Test for clastogenicity in mammalian cells

Report:	II A 5.4.2/1 Schulz M., Landsiedel R. 2011(a) Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) - In vitro chromosome aberration assay in V79 cells BASF DocID 2011/1106426
Date of report:	18-Jul-2011
Testing facility:	BASF SE; Ludwigshafen/Rhein; Germany Fed.Rep.
Guidelines:	OECD 473; EPA 870.5375; (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.10
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) (Batch COD-001417; purity 91.9%) was tested *in vitro* for the ability to induce chromosome and numerical aberrations in Chinese Hamster V79 cells in an experiment in the presence and absence of metabolic activation. Dublicate cultures were used per dose and per test condition. Based on the cytotoxicity results of an initial cytotoxicity study, concentrations of 187.5 to 3000 µg/mL (approx. 10 mM) were tested for clastogenic effects with and without metabolic activation with a pulse treatment of 4 hours. The preparation intervals used were 18 h post treatment-begin. Vehicle (water/culture medium) and positive controls (cyclophosphamide (CPP) and ethylmethanesulfonate (EMS) for the experiment with and without metabolic activation, respectively) were included to demonstrate the sensitivity of the test system.
Prior to cell harvest, addition of Colcemid arrested cells in the metaphase. After slide preparation and staining of the cells, at least 200 well spread metaphases per dose and treatment condition (100 for high dose with S9 activation) were analyzed for chromosomal aberrations.

In the absence of S9 mix an increase in the number of aberrant metaphases was observed at 750 µg/mL and 3000 µg/mL (6.5% and 14.5% aberrant metaphases, excl. gaps respectively) exceeding the historical negative control data range (0.0% – 5.5% aberrant metaphases, excl. gaps). Besides, the aberration rate at the intermediate dose of 1500 µg/mL was slightly below the concurrent vehicle control value and within the range of the historical negative control data. The aberration rate at 3000 µg/mL was statistically significantly increased compared to the concurrent vehicle control group.
In the presence of metabolic activation after 4 hours treatment a clear and dose-dependent increase in the number of chromosomally damaged cells was observed at 750 µg/mL, 1500 µg/mL and 3000 µg/mL (7.0%, 8.0% and 16.0% aberrant metaphases, excl. gaps, respectively). In addition, the aberration rate at 3 000.0 µg/mL was statistically significantly increased compared to the concurrent vehicle control group.

In this study, no relevant increase in the number of cells containing numerical chromosomal aberrations was observed in the absence and the presence of metabolic activation.
Under the experimental conditions chosen for this experiment, the conclusion is drawn that Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) is a chromosome-damaging (clastogenic) substance under in vitro conditions using V79 cells in the absence and the presence of metabolic activation.

(BASF DocID 2011/1106426)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na)
Description:	Solid, yellowish
Lot/Batch #:	COD-001417
Purity:	91.9 % (tolerance ± 1%)
Stability of test compound:	The test substance was stable over the study period under the storage conditions (guaranteed until 01 Nov 2013). The homogeneity of the test substance was ensured by mixing prior to preparation of test substance formulations. The stability of the test substance under storage conditions, dissolved in aqueous solution (MEM) was confirmed.
Solvent used:	water, i.e. culture medium (MEM: Minimal Essential Medium)
2. Control Materials:	
Negative control:	Culture medium, with and without S9 mix
Positive control, -S9:	Ethylmethanesulfonate 500 µg/mL
Positive control, +S9:	Cyclophosphamide 0.5 µg/mL
3. Activation:	S9 was produced from male Wistar rats. The rats were induced by daily application of 80 mg/kg b.w Phenobarbital i.p. and ß-naphthoflavone p.o. on three consecutive days and were sacrificed 24 hours after the last administration. The livers were washed with 150 mM KCL solution, minced and processed for centrifugation at 9000g. The supernatant (so called S9 fraction) was portioned and stored at -70 to -80°C. The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	*Concentration*
Sodium phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms: Chinese hamster V79 cells

5. Culture medium: MEM medium with glutamine supplemented with
- 10% (v/v) fetal calf serum (FCS)
- 1% (v/v) penicillin/streptomycin (10000 IU/10000 µg/mL)
- 1% (v/v) amphotericine B (250 µg/mL)

During exposure to the test substance (only 4-hour treatment), MEM medium was used without FCS supplementation.

6. Test conditions:

a) Preliminary toxicity assay: 0, 23.4, 46.9, 93.8, 187.5, 375.0, 750.0, 1500.0 and 3000 µg/mL for 4 and 18 hours exposure time without S9 mix and 4 hours exposure time with S9 mix.

b) Mutation assay:

1st experiment: 187.5, 375.0, **750.0, 1500.0**, and **3000.0** µg/mL with and without metabolic activation. The doses in bold type were evaluated.

2nd experiment: not performed

B. TEST PERFORMANCE:

1. Dates of experimental work: 21-Mar-2011 - 21-Jun-2011

2. Initial cytotoxicity assay: The pretest was performed following the method described for the main experiment. As indication of test substance toxicity cell count and cell attachment (morphology) were determined for dose selection. The cells were prepared at a sampling time of 18 hours (about 1.5-fold cell cycle time) after 4 and 18 hours exposure time without S9 mix and after 4 hours exposure time with S9 mix.

3. Cytogenicity Assay:

Cell treatment:	Cells were exposed to the test substance, solvent or positive control in pulse treatment experiments for 4 hours. The preparation intervals were 18 h post treatment-begin. Duplicate cultures were run for each dose and condition. The cells were incubated in Quadriperm® dishes at 37°C, 5% CO_2 and ≥ 90% humidity. Two chambers of a Quadriperm dish were used for each concentration. For determination of cytotoxicity, additional cell cultures (using 25 cm² plastic flasks) were treated in the same way as in the main experiment. Growth inhibition was estimated by comparing the cell number in the treated groups with the concurrent control.
Spindle inhibition:	1 µg/mL of Colcemid was added to the cultures 2 - 3 hours prior to harvesting.
Cell harvest:	At the end of the incubation time the culture medium was completely removed. For hypotonic treatment, 5 mL of a 0.4% KCl solution (37 °C) was added for about 20 minutes. The cells were fixed by addition of 5 mL methanol/glacial acetic acid (3:1 v/v). The fixative was changed twice.
Slide preparation:	The slides were removed from the Quadriperm chambers, briefly allowed to drip off and passed through a Bunsen burner flame. After drying, the cells were stained with Giemsa and Titrisol. After rinsing and clarifying in Xylene the cover slips were mounted in Corbit-Balsam.
Metaphase analysis:	Slides were coded prior to analysis. As a rule, the first 100 consecutive well spread metaphases of each culture were counted for all test groups, and if cells had 20 - 22 chromosomes, they were analyzed for structural chromosome aberrations. Numerical chromosome aberrations were also recorded. If there is a clear increase in chromosomally damaged cells, the number of metaphases to be analyzed is reduced from the planned 200 mitoses/test group. A mitotic index based on 1000 cells per culture was determined for all evaluated test groups in both experiments.

Definitions:

Structural aberrations

G' and G" *	Chromatid gap and chromosome gap (isochromatid gap)
B' and B" **	Chromatid break and chromosome break
F' and F" **	Chromatid fragment and chromosome fragment
D' and D" **	Chromatid deletion and chromosome deletion
m. A.	Multiple aberrations; Metaphases with 5 or more aberrations excl. gaps
P ***	Disintegration of chromosomal structure (pulverization)
Ex	Exchanges (translocations); these exchange aberrations (Ex) are divided into intrachanges (Int' and Int" *) and interchanges (I' and I" *).
* Data recorded in the raw data but not presented in the report ** Data not presented in the report summarys *** Data presented in the report summary tables without distinction between chromatid or chromosome deletions	

Numerical chromosome aberrations

Aneuploidy (AP)	Metaphases with absent (hypoploid) or additional (hyperploid) chromosomes; only hyperploid methaphases registered.
Polyploidy (PP)	Changes in the number of chromosomes by whole chromosome sets. A special form of polyploid cells are endoreduplications
Endopolyploidy (EP)	Tetraploid metaphases with diplo-chromosomes (products of endomitotic chromosome reduplication)

4. Statistics:

The proportion of metaphases with aberrations was calculated for each group.

A comparison of each dose group with the vehicle control group was carried out using Fisher's exact test for the hypothesis of equal proportions. This test was Bonferroni-Holm corrected versus the dose groups separately for each time and was performed one sided.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A statistically significant, dose-related, and reproducible increase in the number of structural chromosomal aberrations (excl. gaps).
- The proportion of aberrations (excl. gaps) exceeded both the concurrent negative control range and the historical negative control range.
- A test substance is generally considered non-clastogenic in this test system if:
- There was no statistically significant increase in the number of chromosomally damaged cells at any dose above concurrent control frequencies.
- The aberration frequencies were within the historical negative control range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS:

The stability of the test substance under storage conditions throughout the study period was proven. The stability at room temperature in water over a period of 4 hours has been verified analytically (see AIR2 Dossier Document M II 5.4.1/1).

B. PRELIMINARY CYTOTOXICITY ASSAY:

In the initial range-finding cytotoxicity test for the determination of the experimental doses (dose range 23.4 – 3000 µg/mL), the test substance did not exhibit any pronounced toxicity up to the highest recommended dose of 3000 µg/mL (approx. 10 mM) after 4 hours exposure. In the pretest with 18 hours continuous treatment in the absence of S9 mix (not pursued in main experiment), the cell numbers were clearly reduced at 187.5 µg/mL and above.

C. CYTOGENICITY ASSAYS:

Clastogenic mode of action (see Table 5.4/5 and Table 5.4/6)

After a treatment time of 4 hours a statistically significant increase in the number of chromosomally damaged cells was observed, both without S9 mix and after the addition of a metabolizing system.

In detail, in the absence of S9 mix an increase in the number of aberrant metaphases was observed at 750 µg/mL and 3000 µg/mL (6.5% and 14.5% aberrant metaphases, excl. gaps respectively) exceeding the historical negative control data range (0.0% – 5.5% aberrant metaphases, excl. gaps). Besides, the aberration rate at the intermediate dose of 1500 µg/mL (4.5% aberrant metaphases excl. gaps) was slightly below the concurrent vehicle control value and within the range of our historical negative control data. The aberration rate at 3000 µg/mL (14.5% aberrant metaphases excl. gaps) was statistically significantly increased compared to the concurrent vehicle control group.

In the presence of metabolic activation after 4 hours treatment a clear and dose-dependent increase in the number of chromosomally damaged cells was observed at 750 µg/mL, 1500 µg/mL and 3000 µg/mL (7.0%, 8.0% and 16.0% aberrant metaphases, excl. gaps, respectively). In addition, the aberration rate at 3000 µg/mL was statistically significantly increased compared to the concurrent vehicle control group. Due to clearly positive findings at 3000 µg/mL, the analysis of this test group was restricted to 50 metaphases per culture.

The structural chromosome aberration rates of the vehicle control groups were within our historical negative control data range and, thus, fulfilled the acceptance criteria of this study. The increase in the frequencies of structural chromosome aberrations induced by the positive control substances EMS and CPP clearly demonstrated the sensitivity of the test system and of the metabolic activity of the S9 mix employed. The values were within the range of the historical positive control data and, thus, fulfilled the acceptance criteria of this study.

Based on the above findings a second experiment for confirmation of the data was not performed.

Aneugenic mode of action (see Table 5.4/5 and Table 5.4/6)
No relevant increase in the number of cells with changes in the number of chromosomes was demonstrated either without S9 mix or after the addition of a metabolizing system.

Mitotic Index, Cell count and Cell morphology
According to the results of the determination of the mitotic index and cell count, no clear suppression of the mitotic activity was observed in the absence and presence of metabolic activation and no growth inhibition was observed under both experimental conditions (see Table 5.4/4).
Cell attachment (cell morphology) was not influenced at any dose evaluated for structural chromosomal aberrations, except at 3000 µg/mL after metabolic activation where slight changes were observed (slightly reduced attachment/ few cells rounded).

Osmolarity and pH values were not influenced by test substance treatment.
No test substance precipitation in culture medium at the end of exposure period was observed in both experimental parts.
Vehicle and positive controls were all in a range to ensure the validity of the test.

III. CONCLUSIONS

Under the experimental conditions chosen for this experiment, the conclusion is drawn that Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) is a chromosome-damaging (clastogenic) substance under in vitro conditions using V79 cells in the absence and the presence of metabolic activation.

Table 5.4/4 Bacterial gene mutation assay with Reg.No. 88691 - Cytotoxicity

Schedule	Test groups	S9 mix	Cytotoxicity	
Exposure/ preparation time			Cell number %	Mitotic index %
4/18 hrs	Neg. control.	-	100.0	100.0
	187.5 µg/mL	-	93.0	n.d.
	375 µg/mL	-	104.9	n.d.
	750 µg/mL	-	92.3	100.9
	1500 µg/mL	-	97.5	92.6
	3000 µg/mL	-	82.4	76.0
	Pos. control	-	n.t.	60.8
4/18 hrs	Neg. control.	+	100.0	100.0
	187.5 µg/mL	+	110.9	n.d.
	375 µg/mL	+	112.2	n.d.
	750 µg/mL	+	105.5	117.1
	1500 µg/mL	+	106.0	93.3
	3000 µg/mL	+	103.0	88.9
	Pos. control	+	n.t.	62.1

n.d. = not determined; n.t. = not tested

Table 5.4/5 Chromosome aberration test with Reg.No. 88691 without metabolic activation (4 hours treatment, harvest after 18 hours) - Experiment 1

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle	A	100	11.1	12	12.0	5	5.0	0	0.0	0	0.0	0	0.0	8	7.4	0	0.0	0	0.0
	B	100	10.6	9	9.0	6	6.0	2	2.0	0	0.0	0	0.0	6	5.7	0	0.0	0	0.0
	A + B	**200**		**21**	**10.5**	**11**	**5.5**	**2**	**1.0**	**0**	**0.0**	**0**	**0.0**	**14**	**6.5**	**0**	**0.0**	**0**	**0.0**
Reg.No. 88691																			
750 µg/mL	A	100	10.3	5	5.0	2	2.0	0	0.0	0	0.0	0	0.0	2	2.0	0	0.0	0	0.0
	B	100	11.6	16	16.0	11	11.0	5	5.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0
	A + B	**200**		**21**	**10.5**	**13**	**6.5**	**5**	**2.5**	**0**	**0.0**	**0**	**0.0**	**3**	**1.5**	**0**	**0.0**	**0**	**0.0**
1500 µg/mL	A	100	10.6	9	9.0	5	5.0	1	1.0	0	0.0	0	0.0	3	2.9	0	0.0	0	0.0
	B	100	9.5	10	10.0	4	4.0	2	2.0	0	0.0	0	0.0	3	2.9	0	0.0	0	0.0
	A + B	**200**		**19**	**9.5**	**9**	**4.5**	**3**	**1.5**	**0**	**0.0**	**0**	**0.0**	**6**	**2.9**	**0**	**0.0**	**0**	**0.0**
3000 µg/mL	A	100	7.7	21	21.0	16	16.0	14	14.0	4	4.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	100	8.8	20	20.0	13	13.0	6	6.0	5	5.0	0	0.0	1	1.0	0	0.0	0	0.0
	A + B	**200**		**41**	**20.5***	**29**	**14.5****	**20**	**10.0****	**9**	**4.5****	**0**	**0.0**	**1**	**0.5**	**0**	**0.0**	**0**	**0.0**
Positive control EMS																			
500 µg/mL	A	50	8.4	11	22.0	9	18.0	5	10.0	0	0.0	0	0.0	1	2.0	0	0.0	0	0.0
	B	50	4.8	14	28.0	13	26.0	5	10.0	0	0.0	0	0.0	1	2.0	0	0.0	0	0.0
	A + B	**100**		**25**	**25.00****	**22**	**22.0****	**10**	**10.0****	**0**	**0.0**	**0**	**0.0**	**2**	**2.0**	**0**	**0.0**	**0**	**0.0**

* $p \leq 0.05$; ** $p \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferoni-Holm corrections)

Table 5.4/6 Chromosome aberration test with Reg.No. 88691 with metabolic activation (4 hours treatment, harvest after 18 hours) - Experiment 1

	Culture	No. of Meta-phases	Mitotic index	Aberrant cells including gaps		Aberrant cells excluding gaps		Exchange figures (Ex)		Multiple aberrations (m.A.)		Chromosome Disintegrations (P)		Aneuploidy		Polyploidy		Endopolyploidy	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Vehicle DMSO	A	100	14.2	7	7.0	2	2.0	0	0.0	0	0.0	0	0.0	3	2.7	0	0.0	7	6.4
	B	100	15.6	7	7.0	5	5.0	2	2.0	0	0.0	0	0.0	3	2.8	0	0.0	5	4.6
	A + B	**200**		**14**	**7.0**	**7**	**3.5**	**2**	**1.0**	**0**	**0.0**	**0**	**0.0**	**6**	**2.8**	**0**	**0.0**	**12**	**5.5**
Reg. No. 88691																			
750 µg/mL	A	100	18.1	9	9.0	6	6.0	2	2.0	0	0.0	0	0.0	3	2.9	0	0.0	2	1.9
	B	100	16.8	11	11.0	8	8.0	3	3.0	0	0.0	0	0.0	2	1.9	0	0.0	1	1.0
	A + B	**200**		**20**	**10.0**	**14**	**7.0**	**5**	**2.5**	**0**	**0.0**	**0**	**0.0**	**5**	**2.4**	**0**	**0.0**	**3**	**1.4**
1500 µg/mL	A	100	14.6	15	15.0	8	8.0	4	4.0	0	0.0	0	0.0	2	1.9	0	0.0	1	1.0
	B	100	13.2	10	10.0	8	8.0	4	4.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**200**		**25**	**12.5**	**16**	**8.0**	**8**	**4.0**	**0**	**0.0**	**0**	**0.0**	**2**	**1.0**	**0**	**0.0**	**1**	**0.5**
3000 µg/mL	A	50	16.3	8	16.0	6	12.0	5	10.0	0	0.0	0	0.0	0	0.0	2	3.8	0	0.0
	B	50	10.2	10	20.0	10	20.0	5	10.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	A + B	**100**		**18**	**18.0***	**16**	**16.0****	**10**	**10.0****	**0**	**0.0**	**0**	**0.0**	**0**	**0.0**	**2**	**2.0**	**0**	**0.0**
Positive control CCP																			
0.5 µg/mL	A	50	8.5	18	36.0	15	30.0	9	18.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	B	50	10.0	15	30.0	13	26.0	5	10.0	1	2.0	0	0.0	2	3.8	0	0.0	0	0.0
	A + B	**100**		**33**	**33.0****	**28**	**28.0****	**14**	**14.0****	**1**	**1.0**	**0**	**0.0**	**2**	**2.0**	**0**	**0.0**	**0**	**0.0**

* $p \leq 0.05$; ** $p \leq 0.01$ (Fisher's Exact Test (one-sided) with Bonferoni-Holm corrections)

5.4.3 In vitro genotoxicity - Test for gene mutation in mammalian cells

Report:	II A 5.4.3/1 Schulz M., Landsiedel R. 2011(b) Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) - In vitro gene mutation test in CHO cells (HPRT locus assay) BASF DocID 2011/1184494
Date of report:	26-Jul-2011
Testing facility:	BASF SE; Ludwigshafen/Rhein; Germany Fed.Rep.
Guidelines:	OECD 476 (1997); (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.17; EPA 870.5300
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) (Batch COD-001417; purity 91.9 %) was tested *in vitro* for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HPRT locus in Chinese Hamster CHO cells. Two independent sets of experiments were conducted in the presence and absence of metabolic activation. Based on the results of a preliminary cytotoxicity assay concentrations of 187.5 to 3000 µg/mL were used in the original experiment using a 4 hour exposure period with and without metabolic activation, respectively.

In the second experiment concentrations of 187.5 to 3000 µg/mL were used without metabolic activation using a 24 hour exposure and concentrations of 536.0 to 3000 µg/mL were used with metabolic activation using a 4 hour exposure, respectively. Ethylmethanesulfonate (EMS) and methylcholanthrene (MCA) served as positive controls in the experiments without and with metabolic activation, respectively. After the incubation period treatment media were replaced by culture medium and the cells were incubated for about one week for expression of mutant cells. This was followed by a 6 to 8 day incubation of cells in selection medium containing 6-thioguanine.
Cytotoxic effects indicated by reduced cloning efficiencies of below 20 % of the respective vehicle control were not observed, neither in experiments with nor without metabolic activation.
Neither in the original nor in the confirmatory study an increase in the mutant frequency was observed. The positive control substances, however, resulted in a marked increase in mutant frequency.
Based on the results of the study it is concluded that under the conditions of the test Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) does not induce forward mutations in mammalian cells in-vitro.

(BASF DocID 2011/1184494)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na)

Description:	Solid, yellowish
Lot/Batch #.	COD-001417
Purity:	91.9% (tolerance ± 1%)
Stability of test compound:	The test substance was stable over the study period under the storage conditions and is guaranteed until 01 Nov 2013. The homogeneity of the test substance was ensured by mixing prior to preparation of test substance formulations. The stability of the test substance under storage conditions, dissolved in aqueous solution was confirmed.
Solvent used:	water, i.e. culture medium

2. Control Materials:

Negative control:	The negative controls with and without S9 mix were treated in parallel to the other treatment groups, but only culture medium without test substance was used.
Positive control -S9:	Ethyl methanesulfonate (EMS) 300 µg/mL
Positive control +S9:	Methylcholantrene (MCA) 20 µg/mL

3. Activation:

S9 was produced from male Wistar rats. The rats were induced by daily application of 80 mg/kg b.w. phenobarbital i.p. and ß-naphthoflavone p.o. on three consecutive days and were sacrificed 24 hours after the last administration. The livers were washed with 150 mM KCL solution, minced and processed for centrifugation at 9000g. The supernatant (so called S9 fraction) was portioned and stored at -70 to -80°C.

The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	*Concentration*
Sodium phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organism:

Chinese hamster CHO cells. Stocks of the CHO cell line were maintained at -196°C in liquid nitrogen. Each batch used for mutagenicity testing was checked for mycoplasma contamination. The week prior to treatment, spontaneous HPRT-deficient mutants were eliminated from the stock cultures by growing the cells for 3 to 4 days in pretreatment medium (see below).

5. Culture media:

Culture medium : Ham's F12 medium with stable glutamine and hypoxanthine supplemented with 10% (v/v) fetal calf serum (FCS)

Pretreatment medium: ("HAT" medium): FCS-supplemented Ham's F12 medium with glutamine containing per mL 13.6 µg Hypoxanthine, 0.18 µg Aminopterin, 3.88 µg Thymidine and 10% (v/v) fetal calf serum (FCS).

Selection medium: ("TG" medium): Glutamine- and FCS-supplemented, hypoxanthine-free Ham's F12 medium with 6-thioguanine at a final concentration of 10 µg/mL

All media were supplemented with
- 1% (v/v) penicillin/streptomycin (10000 IU / 10000 µg/mL)
- 1% (v/v) amphotericine B (250 µg/mL)

During exposure to the test substance, Ham‘s F12 medium was used without FCS supplementation. In the case of continuous treatment Ham‘s F12 medium with FCS supplementation was used.

6. Locus examined:

hypoxanthine-guanine-phosphoribosyl transferase (H(G)PRT)

7. Test concentrations:

a) Preliminary toxicity assay:	Nine concentrations ranging from 11.7 to 3000 µg/mL
b) Mutation assay:	
1st experiment:	187.5, 375.0, 750.0, 1500.0 and 3000 µg/mL without and with metabolic activation
2nd experiment:	187.5, 375.0, 750.0, 1500.0 and 3000 µg/mL without metabolic activation, 536.0, 1071.0, 2143.0 and 3000 µg/mL with metabolic activation

B. TEST PERFORMANCE:

1. Dates of experimental work: 15-Jun-2011 to 18-Aug-2011

2. Preliminary cytotoxicity assay:

Cytotoxicity was assessed by determination of the cloning efficiency. About 200 cells were incubated in 25-cm² flasks with 9 test substance concentrations in serum-free Ham's F12 medium for about 4 hours (with and without metabolic activation) or 24 hours (only without metabolic activation) after an attachment period of 20-24 hours. At the end of the exposure period, the cells were washed with Hanks' balanced salt solution (HBSS), covered with Ham's F12 and incubated for a further 5 - 8 days. After this incubation period, colonies were fixed, stained and counted. Besides the cloning efficiency the following additional parameters were measured: pH, osmolarity and the determination of precipitates (solubility).

3. Mutation Assay:

Pretreatment of Cells:	Cells with a passage number ≥ 2 after thawing from the frozen cells stock were seeded into 75 cm^2-flasks and incubated with "HAT" medium during the week prior to treatment to eliminate spontaneous HPRT-deficient mutants. Afterwards, a passage into culture medium followed and the cells were incubated for further 3-4 days.
Cell treatment:	For each test group, about $1x10^6$ cells per flask were seeded into 175 cm^2 flasks containing about 20 mL Ham's F12 medium supplemented with 10% FCS and incubated for about 20 - 24 hours with 5% CO_2 at 37°C and > 90% humidity for cell attachment. 2 flasks were used for each test group. After the cell attachment period the medium was replaced by the treatment medium. In case of experiments without metabolic activation the treatment medium consisted of 18 mL Ham's F12 medium without FCS plus 2 mL positive control or test substance preparation, respectively. In case of metabolic activation the treatment medium consisted of 14 mL Ham's F12 medium without FCS, 2 mL positive control or test substance preparation and 4 mL S9-mix, respectively. Concurrent negative and positive controls were tested in parallel. The cells were exposed for 4 hours both with and without S9-mix (or for 24 hours without S9-mix in the second experiment) at 5% CO_2, 37°C and ≥ 90% humidity.

Expression:	After incubation for 4 or 24 hours, respectively, the serum-free treatment medium was replaced by 20 mL Ham's F12 medium with 10% FCS after having been rinsed several times with Hanks' balanced salt solution (HBSS). The following 1st passage was carried out after an incubation period of about 3 days following the 4 hour exposure or 2 days following the 24 hour exposure period. After an entire expression period of 6 - 8 days the cells were transferred into the selection medium (2nd passage)
Selection:	For the mutant selection, six 75-cm^2 flasks each were seeded with $3x10^5$ cells from each treatment group in selection medium (TG medium) and incubated for about 6 to 8 days. At the end of the selection period, colonies were fixed with methanol, stained with Giemsa and counted.
Determination of Cytotoxicity:	Cloning efficiency 1 (survival): The survival (cloning efficiency 1; CE1) was determined in parallel to the mutagenicity test. Approximately 200 cells per dose group were seeded into duplicate 25 cm² flasks using 5 mL Ham`s F12 medium with 10 % FCS. After a 20-24 hour attachment period the cells were incubated with test substance, vehicle or positive control for 4 or 24 hours as described above. At the end of the treatment period the cells were washed with HBSS and the serum-free treatment medium was replaced by Ham's F12 medium with 10% FCS. After a further incubation for about 5 to 8 days the colonies were fixed, stained and counted. Cloning efficiency 2 (viability): The viability (cloning efficiency 2; CE2) was determined in parallel to the selection of mutants under the same conditions as described for cloning efficiency 1.

Calculations:

Mutant frequency: Uncorrected mutant frequency:

$$MF_{uncorr} = \frac{\text{total number of mutant colonies}}{\text{number of seeded cells}} \times 10^6$$

Corrected mutant frequency:

$$MF_{corr} = \frac{MF_{uncorr}}{CE_{2\,absolute}} \times 100$$

Cloning efficiency (CE,%) absolute:

$$CE_{absolute} = \frac{\text{total number of colonies in the test group}}{\text{total number of seeded cells in the test group}} \times 100$$

relative, in comparison to control:

$$CE_{relative} = \frac{\text{CE of the dose group}}{\text{CE of the vehicle control}} \times 100$$

4. Statistics:

Due to the clearly negative findings, a statistical evaluation was not carried out.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- Increases of the corrected mutation frequencies ($MF_{corr.}$) both above the concurrent vehicle control values and the historical negative control range.
- Evidence of reproducibility of any increase in mutant frequencies.
- A statistically significant increase in mutant frequencies and the evidence of a dose-response relationship. Isolated increases of mutant frequencies above the historical negative control range (i.e. 15 mutants per 10^6 clonable cells) or isolated statistically significant increases without a dose-response relationship may indicate a biological effect but are not regarded as sufficient evidence of mutagenicity.

A test substance is generally considered negative in this test system if:

- The corrected mutation frequency ($MF_{corr.}$) in all dose groups is within the historical control range and is not significantly above the concurrent negative control

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance at room temperature in water over a period of 4 hours was analytically verified (see AIR2 Dossier Document M II 5.4.1/1).

B. PRELIMINARY CYTOTOXICITY ASSAY

In the preliminary experiment without metabolic activation a relative cloning efficiency ($CE_{relative}$) of 50.6 % was observed at 3000 µg/mL after 4 hours treatment and 51.6 % at 1500 µg/mL after 24 hours treatment. At the next higher test concentration the $CE_{relative}$ sank to 21.6 %.

In the preliminary experiment with metabolic activation $CE_{relative}$ varied between 81.9 and 95.0 % at concentrations of 11.7 and 3000 µg/mL, respectively.

No precipitation of the test substance was noted at any concentrations with and without metabolic activation. No marked effects on pH and osmolarity were observed.

Based on these data the highest concentration tested in the first mutagenicity experiment was 3000 µg/mL without and with metabolic activation.

C. MUTAGENICITY ASSAYS

In this study, no relevant increase in the number of mutant colonies was observed either without S9 mix or after the addition of a metabolizing system. In both experiments after 4 and 24 hours treatment with the test substance the values for the corrected mutation frequencies ($MF_{corr.}$: 0.00 – 8.17 per 10^6 cells) were close to the respective vehicle control values ($MF_{corr.}$: 1.03 – 3.57 per 10^6 cells) and clearly within the range of the historical negative control data (without S9 mix: $MF_{corr.}$: 0.00 – 15.95 per 10^6 cells; with S9 mix: $MF_{corr.}$: 0.00 – 15.68 per 10^6 cells).
The positive control substances EMS (without S9 mix; 300 µg/mL) and MCA (with S9 mix; 20 µg/mL) induced clearly increased mutant frequencies as expected. The values of the corrected mutant frequencies (without S9 mix: $MF_{corr.}$: 76.44 – 352.70 per 10^6 cells; with S9 mix: $MF_{corr.}$: 53.15 – 87.14 per 10^6 cells) were clearly within the historical positive control data range (without S9 mix: $MF_{corr.}$: 48.83 – 1 338.10 per 10^6 cells; with S9 mix: $MF_{corr.}$:26.29 – 413.54 per 10^6 cells), thus, fulfilled the acceptance criteria of this study.

Details are given in Table 5.4/7 and Table 5.4/8.

Table 5.4/7 Gene mutation in mammalian cells - 1st experiment

Test group	Number of mutant colonies[a]	Mutant frequency (per 10^6 cells)		CE_1 (survival) (4h after treatment; approx. 200 cells/flask seeded		CE_2 (viability) (after the expression period; approx. 200 cells/flask seeded)	
				Cloning efficiency (%)		Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	relative
Without metabolic activation; 4-hour exposure period							
Vehicle (Culture medium)	3	0.83	1.03	80.3	100.0	86.8	100.0
Reg.No 88691							
187.5µg/mL	n.c.	--	--	89.1	111.1	n.c.	n.c.
375.0 µg/mL	5	1.39	1.59	91.5	114.0	86.8	100.0
750.0 µg/mL	4	1.11	1.35	91.8	114.3	81.9	94.4
1500.0 µg/mL	3	0.83	0.85	85.3	106.2	88.4	101.9
3000.0 µg/mL	3	0.83	0.95	76.8	95.6	89.8	103.5
Positive control EMS							
300.0 µg/mL	246	68.33	76.44	81.5	101.6	89.5	103.2
With metabolic activation; 4-hour exposure period							
Vehicle (Culture medium)	9	2.5	2.88	88.9	100.0	87.0	100.0
Reg.No. 88691							
187.5µg/mL	n.c.	--	--	90.8	102.1	n.c.	n.c.
375.0 µg/mL	5	1.39	1.73	90.5	101.8	85.9	98.7
750.0 µg/mL	7	1.94	2.46	99.0	111.4	87.1	100.1
1500.0 µg/mL	7	1.94	2.09	92.5	104.1	90.1	103.6
3000.0 µg/mL	0	0.00	0.00	95.8	107.7	89.0	102.3
Positive control MCA							
20.0 µg/mL	152	42.22	53.15	93.1	104.8	79.4	91.2

[a] number of colonies approx 7 days after seeding 300,000 cells/flask into selection medium (sum of 12 plates)
[b] correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period
n.c. culture was not continued since a minimum of only 4 analysable concentrations is required.

Table 5.4/8 Gene mutation in mammalian cells - 2nd experiment

Test group	Number of mutant colonies[a]	Mutant frequency (per 10^6 cells)		CE_1 (survival) (4h after treatment; approx. 200 cells/flask seeded		CE_2 (viability) (after the expression period; approx. 200 cells/flask seeded)	
				Cloning efficiency (%)		Cloning efficiency (%)	
		Non corrected	Corrected[b]	absolute	relative	absolute	Relative
Without metabolic activation; 24-hour exposure period							
Negative (Culture medium)	10	2.78	3.57	76.8	100.0	78.0	100.0
Reg.No 88691							
187.5µg/mL	n.c.	--	--	69.3	90.2	n.c.	n.c.
375.0 µg/mL	23	6.39	8.17	84.9	110.6	78.3	100.3
750.0 µg/mL	10	2.78	3.92	67.8	88.3	72.1	92.5
1500.0 µg/mL	3	0.83	1.21	54.6	71.2	68.1	87.3
3000.0 µg/mL	7	1.94	2.48	37.4	48.7	80.3	102.9
Positive control EMS							
300 µg/mL	842	233.89	352.70	62.1	80.9	66.1	84.8
With metabolic activation; 4-hour exposure period							
Vehicle (Culture medium)	7	1.94	2.31	78.0	100.0	83.8	100.0
Reg.No 88691							
536.0 µg/mL	22	6.11	8.14	90.3	115.7	75.9	90.6
1071.0 µg/mL	5	1.39	1.60	92.6	118.8	85.6	102.2
2143.0 µg/mL	10	3.33	4.51	89.3	114.4	73.9	88.2
3000.0 µg/mL	11	3.06	3.75	83.8	107.4	78.4	93.6
Positive control MCA							
20 µg/mL	232	64.44	87.14	92.3	118.3	74.0	88.4

[a] number of colonies 7 days after seeding 300,000 cells/flask into selection medium (sum of 12 plates)
[b] correction on the basis of absolute cloning efficiency 2 (viability) at the end of the expression period
n.c. culture was not continued since a minimum of only 4 analysable concentrations is required.

In both experiments in the absence and presence of S9 mix no cytotoxicity indicated by reduced relative cloning efficiency of about or below 20% relative survival was observed up to the highest applied test substance concentration.

The pH and osmolarity of the incubations as well as the solubility of the test substance were not altered at the concentrations tested. Test substance precipitation did not occur up the highest tested concentration of 3000 µg/mL.

III. CONCLUSIONS

Based on the results of the study it is concluded that under the conditions of the test Reg.No.88691 (Bentazone-sodium, BAS 351 H-Na) does not induce forward mutations in the HPRT locus in CHO cells in vitro.

5.4.4 In vivo genotoxicity (somatic cells) - Bone marrow or micronucleus

Report:	II A 5.4.4/1 [redacted] 2011(c) Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) - Micronucleus test in bone marrow cells of the mouse BASF DocID 2011/1184970
Date of report:	29-Sep-2011
Testing facility:	[redacted]
Guidelines:	OECD 474 (1997); (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12; EPA 870.5395
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	II A 5.4.4/2 [redacted] 2011(e) Amendment No. 1 - Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) - Micronucleus test in bone marrow cells of the mouse BASF DocID 2011/1277971
Date of report:	21-Dec-2011
Testing facility:	[redacted]
Guidelines:	OECD 474 (1997); (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.12; EPA 870.5395
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Note:	This Amendment provides the clinical observations of the pretest in tabular form and the correct Certificate of Analysis.

Executive Summary

Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) (batch: COD-001417, purity: 91.9%) was tested for chromosomal damage (clastogenicity) and for the ability to induce spindle poison effects (aneugenic activity) in male and female Crl:NMRI mice using the micronucleus test method. For this purpose, the test substance dissolved in water was administered once orally to groups of 5 male mice at dose levels of 340, 680 and 1360 mg/kg (approx. 312.5, 625, and 1250 mg/kg bw of active ingredient) and to female mice at dose levels of 217.5, 435 and 870 mg/kg body weight (approx. 200, 400 and 800 mg/kg bw of active ingredient) in a volume of 10 mL/kg body weight. The dose levels were calculated as pure active ingredient equivalents. The vehicle water served as negative and cyclophosphamide and vincristine sulfate as positive controls. The animals were sacrificed 24 hours or 48 hours (additional high dose group) after the last administration and the bone marrow of the two femora was prepared. After staining of the preparations, 2000 polychromatic erythrocytes were evaluated per animal and investigated for micronuclei. The normocytes with and without micronuclei occurring per 2000 polychromatic erythrocytes were also recorded.
According to the results of the present study single oral administration of Reg.No. 88691 did not lead to a relevant increase in the number of polychromatic erythrocytes containing either small or large micronuclei. In male mice a statistically significant increase compared to the concurrent vehicle control was observed after exposure to 1 250 mg/kg body weight at 24-hour sacrifice interval. Females showed a dose-related increase (without statistical significance) of micronucleated polychromatic erythrocytes. However, the rate of micronuclei in this study was close to the range of the concurrent vehicle controls in all dose groups and at all sacrifice intervals and within the range of the historical vehicle control data.

The administration of the test substance in the pretest led to severe toxicity in males and mortality in females. At the respective lower dose levels used in the main study, weak signs of toxicity in male and female animals were observed in the highest administered dose. The systemic bioavailability upon oral administration is thereby demonstrated. Additionally, the plasmakinetic data demonstrate a high bioavailability of the active ingredient after oral administration in all tested species (rat, mouse and rabbit).
Both of the positive control chemicals, i.e. cyclophosphamide for clastogenic effects and vincristine sulphate for induction of spindle poison effects, led to the expected increase in the rate of polychromatic erythrocytes containing small (cyclophosphamide) or small and large (vincristine sulphate) micronuclei, thus demonstrating the sensitivity of the test system.

Thus, under the experimental conditions of this study, the test substance Reg.No. 88691 does not induce cytogenetic damage in bone marrow cells of Crl:NMRI mice in vivo.

(BASF DocID 2011/1184970 & 2011//1277971)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na)
Description:	Solid, yellowish
Lot/Batch #:	COD-001417
Purity:	91.9% (tolerance ±1%)
Stability of test compound:	The stability of the test substance under storage conditions is guaranteed until 01 November 2013. The stability of the test substance dissolved in water over 1 hour was confirmed. Homogeneity of the preparations was ensured by mixing.
Solvent used:	Deionized water
2. Control Materials:	
Negative and solvent control:	Deionized water
Positive control:	Cyclophosphamide (CCP) 20 mg/kg for the determination of clastogenic effects, gavage administration Vincristine sulphate (VCR) 0.15 mg/kg for the determination aneugenic effects, intraperitoneal administration
3. Test animals:	
Species:	Albino mice
Strain:	Crl:NMRI
Sex:	Male and female for the main study; male and female for the range finding study
Age:	5 - 8 weeks
Weight at beginning of study:	26.8 g (mean)
Source:	Charles River Laboratories Germany GmbH
Number of animals per dose:	
Range finding study:	Not indicated in the report
Micronucleus assay:	5/sex/dose
Acclimation period:	At least 5 days
Diet:	Rat - Mouse - Hamster Diet (Provimi Kliba SA, Kaiseraugst, Switzerland), ad libitum
Water:	Tap water in bottles ad libitum
Housing:	During the study the mice were housed individually in Makrolon cages, type MI Dust-free wooden bedding was used in this study

4. Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	30% - 70%
Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound concentration:

Range finding test: up to 1500 mg/kg

Micronucleus assay: 312.5; 625 and 1250 mg active ingredient/kg bw for males
200, 400 and 800 mg active ingredient /kg bw for females

Regarding the purity of the test substance respective higher concentrations were formulated in the vehicle:
340, 680 and 1360 mg test substance/kg bw for males
217.5, 435 and 870 mg test substance/kg bw for females

The test substance was administered once by oral gavage using an application volume of 10 mL/kg.

B. TEST PERFORMANCE

1. Dates of experimental work: 09-Jun-2011 to 24-Aug-2011

2. Preliminary range finding test:

Male and female NMRI mice were administered the test substance by oral gavage at a dose of up to 1500 mg/kg bw.

3. Micronucleus test:

Treatment and sampling:	Groups of 5 mice were treated once with either the vehicle or the test substance by oral gavage in an application volume of 10 mL/kg bw and the volume to be applied was calculated based on actual weight on the first day of administration. The test substance dose levels in males were 312.5, 625 or 1250 mg Reg.No. 88691/kg bw, for females the respective dose levels were 200, 400 or 800 mg/kg. The positive control substances were administered once: CCP by oral gavage; VCR by i.p. injection. The animals were surveyed for evident clinical signs of toxicity throughout the study. The animals were sacrificed 24 hours (all test substance concentrations, vehicle, both positive controls) and 48 hours (highest test substance concentration, vehicle) after the treatment, respectively. and the two femora were prepared free of all soft tissue. After cutting the epiphyses the bone marrow was flushed out in a centrifugation tube with fetal calf serum and subsequently centrifuged at 300 g for 5 minutes. The supernatant was discharged and the pellet resuspended in about 50 µl fresh fetal calf serum (FCS).
Slide preparation:	One drop of the suspension was applied on a clean microscopic slide and smears were prepared. After air drying the smears were stained with eosin and methylene blue (modified May Grünwald of Wrights solution), rinsed, and finally stained in 7.5% Giemsa solution. After rinsing and clarifying in xylene, the preparations were mounted. The slides were coded prior to microscopic evaluation.
Slide evaluation:	In general, 2000 polychromatic erythrocytes (PCEs) from each male or female animal of every test group were evaluated and investigated for micronuclei (MN). The normochromatic erythrocytes (NCEs) that occurred were also scored. The increase in the number of micronuclei in polychromatic erythrocytes of treated animals as compared to the solvent control group provides an index of a chromosome-breaking (clastogenic) effect or of a spindle activity of the substance tested. In addition the number of small micronuclei (d < D/4) and of large micronuclei (d > D/4) (d = diameter of micronucleus, D = cell diameter) was determined: The size of micronuclei may indicate the possible mode of action of the test substance, i.e. a clastogenic or a spindle poison effect. The ratio of polychromatic to normochromatic erythrocytes was calculated. An alteration of this ratio indicates a toxic effect on erythropoiesis and thus, that the test substance actually reached the target organ.

4. Statistics:

The number of polychromatic erythrocytes with micronuclei was analyzed by comparing the dose groups with the vehicle control using the Mann-Whitney U-test (modified rank test according to Wilcoxon). Here, the relative frequencies of cells with micronuclei of each animal were used.

5. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and significant increase in the number of micronucleated polychromatic erythrocytes was observed.
- The proportion of cells containing micronuclei exceeded both the values of the concurrent negative control range and the negative historical control range.

A test substance is generally considered negative in this test system if:

- There was no significant increase in the number of micronucleated polychromatic erythrocytes at any dose above concurrent control frequencies.
- The frequencies of cells containing micronuclei were within the historical control range.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The stability of the test substance at room temperature in the vehicle (deionized water) over a period of 4 hour was verified analytically (data see AIR2 Dossier Document M II 5.4.1/1) and additionally confirmed within the study over the period of substance administration (1 hour). The homogeneity of the test substance in the vehicle was guaranteed by constant stirring during the removal and administration of the test substance formulation and by analytical determination of 3 individual samples of each concentration. The mean concentrations were determined as 97 to 104% for the doses used in males and 96 – 111% for the doses used in females, thus remaining essentially within the expected range of 90 – 110 %.

B. PRELIMINARY RANGE FINDING TEST

In a pretest to determine the acute oral toxicity in both sexes, females died from 1000 mg/kg body weight active ingredient (1100 mg/kg bw test substance) onward. Male animals showed severe signs of toxicity at the highest applied dose of 1500 mg/kg body weight active ingredient. The clinical signs observed were piloerection, hunched posture and reduced general condition. Thus, male and female animals were used in the main experiment as requested by the current OECD Guideline 474.

C. CLINICAL EXAMINATIONS

No signs of toxicity were observed in the vehicle or positive control groups. In the test compound treated animals the two lower dose groups were also free of clinical signs, yet weak signs of toxicity (piloerection from 1 hour to 1 day post-dosing) were noted in both sexes of the respective high doses.

D. MICRONUCLEUS ASSAY (data see Table 5.4/9 and Table 5.4/10)

Treatment of males at the highest dose of 1250 mg/kg body weight led to 2.7‰ polychromatic erythrocytes containing micronuclei after 24 hours ($p \leq 0.05$) and 2.0‰ after 48 hours. In the two lower dose groups, rates of micronuclei of 3.0‰ (625 mg/kg group) and 2.0‰ (312.5 mg/kg group) were detected after a sacrifice interval of 24 hours in each case. All values were slightly above the concurrent control values (1.4‰ after the 24 hour- and 1.7‰ after the 48-hour sacrifice) but within the historical negative control data range (0.4 – 2.7‰) and, therefore, and due to the missing dose response, even the statistically significant high dose value has to be regarded as biologically irrelevant.

In females, the single oral administration of the highest dose of 800 mg/kg body weight led to 2.7‰ polychromatic erythrocytes containing micronuclei after 24 hours and 2.0‰ after 48 hours. In the two lower dose groups, rates of micronuclei of 1.7‰ (400 mg/kg group) and 1.5‰ (200 mg/kg group) were detected after a sacrifice interval of 24 hours. The values of this apparent dose related increase in the 24 hour sacrifice group (1.5‰, 1.7‰ and 2.7‰) are only partially above the 24 hour and 48 hour sacrifice-concurrent control of each 1.8‰ and clearly within the historical negative control data range (0.4 – 2.7‰) and, therefore, this observation has to be regarded as biologically irrelevant.

The number of normochromatic or polychromatic erythrocytes containing small micronuclei ($d < D/4$) or large micronuclei ($d \geq D/4$) did not distinctly deviate from the vehicle control values at any of the sacrifice intervals and in any gender and was within the historical vehicle control data range (0.0-1.0‰ for large Micronuclei). Thus, additionally, the increase in males is considered biologically not relevant.

The PCE/NCE ratio was not affected by treatment with the test substance. There was no relevant inhibition of erythropoiesis induced by the treatment with Bentazone-sodium. However, the systemic distribution of BAS 351 H and its bioavailability upon oral administration is demonstrated by the induced signs of systemic toxicity observed in the high dose group in males and females of this study. Additionally, several plasmakinetic studies proved systemic distribution and the high bioavailability of bentazone after oral administration in all tested species.

The positive control substance for clastogenicity, cyclophosphamide, led to a statistically significant increase in males and females (16.4 and 13.4‰, respectively) in the number of polychromatic erythrocytes containing mainly small micronuclei, as expected.
Vincristine sulfate, a spindle poison, produced a statistically significant increase in males and females (36.8‰ and 48.8‰) in the number of polychromatic erythrocytes containing micronuclei. A significant portion increase, 10.3‰ and 16.1‰, was attributable in males and females to large micronuclei. The positive controls thus demonstrated the sensitivity of the test system.

Table 5.4/9 Micronucleus test in male mice administered Reg.No. 88691 by oral gavage

Treatment	PCE				NCE		PCE/NCE ratio
	scored	With MN			Number	With MN [‰]	
		total [‰]	small [‰]	large [‰]			
Deionized water, 24 h	10000	**1.4**	1.4	0.0	5573	1.4	1.79
Reg.No 88691#,							
312.5 µg/mL, 24 h	10000	**2.0**	1.9	0.1	5435	0.7	1.84
625 µg/mL, 24 h	10000	**3.0**	2.8	0.2	6014	2.3	1.66
1250 µg/mL, 24 h	10000	**2.7***	2.2	0.5*	5535	2.5	1.81
Positive controls							
Cyclophosphamide	10000	**16.4****	16.3**	0.1	5105	1.4	1.96
Vincristine	10000	**36.8****	26.5**	10.3**	5480	2.0	1.82
Deionized water, 48 h	10000	**1.7**	1.6	0.1	4842	0.8	2.07
Reg.No 88691#, 1250 µg/mL, 48 h	10000	**2.0**	1.7	0.3	5611	1.1	1.78

* $p \leq 0.05$ ** $p \leq 0.01$ (Wilcoxon Test; one sided)
PCE: polychromatic erythrocytes; NCE: normochromatic erythrocytes; MN: micronuclei
concentration is given as amount of pure Bentazone-sodium

Table 5.4/10 Micronucleus test in female mice administered Reg.No. 88691 by oral gavage

Treatment	PCE				NCE		PCE/NCE ratio
	scored	With MN			Number	With MN [‰]	
		total [‰]	small [‰]	large [‰]			
Deionized water	10000	**1.8**	1.6	0.2	4180	1.4	2.39
Reg.No 88691#,							
200 µg/mL	10000	**1.5**	1.4	0.1	5182	0.6	1.93
400 µg/mL	10000	**1.7**	1.7	0.0	4772	1.5	2.10
800 µg/mL	10000	**2.7**	2.5	0.2	5058	1.8	1.98
Positive controls							
Cyclophosphamide	10000	**13.4****	13.2**	0.2	4768	1.0	2.10
Vincristine	10000	**48.8****	32.7**	16.1**	5891	0.8	1.70
Deionized water, 48 h	10000	**1.8**	1.7	0.1	5102	0.8	1.96
Reg.No 88691#, 1250 µg/mL, 48 h	10000	**2.0**	2.0	0.0	4417	1.6	2.26

** $p \leq 0.01$ (Wilcoxon Test; one sided)
PCE: polychromatic erythrocytes; NCE: normochromatic erythrocytes; MN: micronuclei
concentration is given as amount of pure Bentazone-sodium

III. CONCLUSIONS

Under the experimental conditions of this study, the test substance Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) has no chromosome-damaging (clastogenic) effect nor does it lead to any impairment of chromosome distribution in the course of mitosis (aneugenic activity) in bone marrow cells of male and female NMRI mice in vivo.

5.4.5 In vivo genotoxicity (somatic cells) - DNA repair or mouse spot tests

Report: II A 5.4.5/1
[redacted] 2011(d)
Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) - In vivo unscheduled DNA synthesis (UDS) assay in rat hepatocytes
BASF DocID 2011/1192857
Date of report: 16-Nov-2011
Testing facility: [redacted]
Guidelines: OECD 486; (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.39
GLP: Yes
(laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report: II A 5.4.5/2
[redacted] 2011(f)
Amendment No. 1- Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) - In vivo unscheduled DNA synthesis (UDS) assay in rat hepatocytes
BASF DocID 2011/1277972
Date of report: 21-Dec-2011
Testing facility: [redacted]
Guidelines: OECD 486; (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.39
GLP: Yes
(laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)
Note: This Amendment provides the clinical observations of the pretest in tabular form, including the dose administered of the test item, and the Certificate of Analysis which was not attached to the original Report.

Executive Summary

Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) (batch: COD-001417, purity: 91.9%) was assessed for its potential to induce DNA repair synthesis in hepatocytes of Wistar rats in vivo at 3-hour and 14-hour sampling time. The test substance, dissolved in water, was administered once orally to 4 (respectively 6 in the vehicle control) male animals at dose levels of 275, 550 and 1100 mg/kg body weight (approx. 250, 500 and 1000 mg/kg bw of active ingredient) in a volume of 10 mL/kg bw.
Animals treated with the vehicle only served as negative control. As positive control, 2-acetylaminofluoren (2-AAF) was administered at a dose of 50 mg/kg bw. Hepatocytes were harvested 3 and 14 hours after administration.
Due to unexpected death in the high dose group, and in addition, one died animal in the mid dose group without further clinical signs of toxicity, an additional main test, designated 2nd Experiment, was performed with administration of 275 mg/kg body weight. No UDS assessment of the 1100 mg/kg bw. animals could be made.

Neither in hepatocytes harvested 3 hours nor harvested 14 hours after a single oral administration of Reg.No. 88691 a relevant increase in the mean net nuclear grain counts or in the percentage of cells in repair was noted at any dose. In both main experiments no reduced viability of hepatocytes as indication for test substance induced toxicity was observed. In contrast, 2-AAF treatment led to a marked increase in the number of net nuclear grains as well as the percentage of cells in repair, thus demonstrating the sensitivity of the test system.
The bioavailability of the test substance in the rat was not directly assessed in the study but can be deduced from the signs of toxicity and in general from the plasmakinetic studies performed with Bentazone as free acid or sodium salt, in which test substance in the liver after oral administration to rats have been demonstrated.
Thus, under the experimental conditions of this study, the test substance Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) does not induce DNA-damage leading to increased unscheduled DNA synthesis in hepatocytes of male Wistar rats in vivo.

(BASF DocID 2011/1192857 & 2011/1277972)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:	Reg.No. 88691 (Bentazone-sodium, BAS 531 H-Na)
Description:	Solid, yellowish
Lot/Batch #:	COD-001417
Purity:	91.9 % (tolerance ± 1.0%)
Stability of test compound:	The stability of the test substance under storage conditions is guaranteed until 01 November 2013. The stability of the test substance dissolved in water over 1 hour was confirmed.
Homogeneity:	The homogeneity of the test substance was ensured by mixing before preparation of the test substance preparations.
Solvent used:	Deionized water for test substance, Corn oil for positive control

2. Control Materials:	
Negative:	A negative control was not employed in this study
Solvent control:	Deionized water
Positive control:	2-acetylaminofluorene (2-AAF), 50 mg/kg, suspended in corn oil

3. Test animals:	
Species:	Albino rats
Strain:	Wistar Han (Crl:WI (Han))
Sex:	Male
Age:	8 - 10 weeks
Weight at beginning of study:	Mean body weight 242.9 g (± 11.80 g)
Source:	Charles River Laboratories Deutschland GmbH
Number of animals per dose:	3 +1 males/dose for test and pos control groups, 4 + 2 males for vehicle control group
Acclimation period:	at least 5 days
Diet:	Maus/Ratte Haltung "GLP" (Provimi Kliba SA, Kaiseraugst, Switzerland), ad libitum
Water:	Tap water in bottles ad libitum
Housing:	Individual housing in Makrolon type III cages. Dust free wooden bedding was used in this study.

4. Environmental conditions:

Temperature:	20 - 24 °C
Humidity:	30% -70%
Air changes:	frequency not indicated
Photo period:	12-hour light-dark cycle (06:00 - 18:00, 18:00 - 06:00)

5. Test compound dose: 275, 550 and 1100 mg/kg bw (approximately 250, 500 and 1000 mg/kg bw active ingredient)

6. Perfusion and culture media:

Perfusion solution:	100 mL Ca^{2+} and Mg^{2+}-free Hanks balanced salt solution (HBBS) buffered with 1 mL HEPES (4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid) plus 18 mg ethylene glycol-bis(ß-amino ethyl ether)N,N,N',N'-tetraacetic acid (EGTA) solution (0.5 mM) plus 0.1 mL gentamycin sulfate (50 mg/mL).
Collagenase perfusion:	WMEI buffered with HEPES buffer (1M), NaOH plus collagenase-IV (50,000 Units)
Incubation medium:	(= Williams medium E incomplete (WMEI)): Williams medium E supplemented with glutamine and gentamycin sulfate
Attachment medium:	(= Williams medium E, complete (WMEC)): WMEI with 10% (v/v) fetal calf serum (FCS)
Labeling medium:	WMEI supplemented with 1% (v/v) 3H-thymidine (final concentration about 0.37 MBq/mL) and 1% (v/v) HEPES buffer
Unlabelled Thymidine solution:	100 mL WMEI with 6.1 mL thymidine

B. TEST PERFORMANCE

1. Dates of experimental work: 27-Jun-2011 - 17-Oct-2011

2. Preliminary range finding test:
In a pretest for the determination of the acute oral toxicity, deaths in males and females were observed down to a dose of 1360 mg/kg bw. Bentazone-sodium (approx. 1250 mg/kg bw active ingredient Bentazone-acid). However, the surviving animals of both genders showed no distinct differences in clinical observations. The clinical signs were piloerection, abdominal position, reduced general condition and shallow respiration. At 1100 mg/kg bw no signs of toxicity were observed.
On account of these results 1100 mg/kg bw (approx. 1000 mg/kg bw. active ingredient) was defined as maximum tolerated dose (MTD) for Bentazone-sodium. Only male animals were used for the main experiments as requested by the current OECD Guideline 486. As further dose 550 mg/kg bw (approx. 500 mg/kg bw. active ingredient) was selected. Due to unexpected deaths in the highest dose group the study was extended with an additional test group of 275 mg/kg body weight (approx. 250 mg/kg bw. active ingredient) at both sacrifice intervals (2nd Experiment).

3. UDS test:

Treatment:	Groups of 3 (+1) male rats each were initially administered the vehicle, 550 and 1100 mg/kg Reg,No. 88691, or 50 mg/kg bw of the positive control substance 2-AAF by oral gavage (1st Experiment). The application volume was 10 mL/kg and the volume administered was calculated based on actual weight on the day of administration. The animals were fasted for 6 h prior to the treatment and surveyed for evident clinical signs of toxicity throughout the study. As there was unexpected mortality, an additional group dosed with 275 mg/kg bw. was added for both sampling times (2nd Experiment). This dose and the 550 mg/kg bw. dose were used for the determination of DNA repair activity.
Isolation of hepatocytes:	Three and 14 hours after treatment each one group of 3 + 1 males per dose level were anesthetized by intraperitoneal injection with Narcoren®, (100 mg/kg bw., 2 mL/kg bw). After opening of the abdominal cavity the livers were perfused with the perfusion medium followed by the collagenase solution. After removal of the livers and isolation of the hepatocytes, the cells were washed with WMEI, mixed and centrifuged at 40 x g for 5 minutes. The supernatant was discharged and the pellet resuspended in WMEI to yield single cell suspensions.
Determination of cell viability:	The viability of the hepatocytes was determined by mean of vital staining (trypan vital dye-exclusion method), and the yield was determined by cell counting in the counting chamber. The amount of viable cells (in %) was calculated as viable cells divided by the sum of viable + dead cells

multiplied with 100. If the viability was clearly below 70%, a replacement animal was used.

Culture of hepatocytes: About $4x10^5$ viable hepatocytes (as determined by trypan blue exclusion) were seeded on coverslips on a 1.9 cm^2 well containing 2 mL of attachment medium (WMEC). 4 - 6 wells were used per animal. After an attachment period of about 2 hours with 5% CO_2 at 37°C and ≥ 90% humidity, the medium (WMEC) was replaced by fresh medium (WMEI) to remove non-adherent cells.

Labeling: The medium (WMEI) was replaced by 2 mL labeling medium, and the cells were incubated for 4 hours. Thereafter, cells were washed with HBSS or WMEI and - after addition of fresh medium containing 0.25 mM unlabeled thymidine - the cells were incubated for at least 12 hours.
Finally, the cells on the coverslips were then fixed with ethanol/acetic acid (3 : 1, v/v) for at least 30 minutes, and air-dried after being rinsed with distilled water.

Autoradiography: After the mounting of the coverslips the slides were covered with a photographic emulsion and stored in the dark at -20°C for at least 3 days and finally the autoradiographs were developed.

Cytotoxicity test: In addition to the determination of viability (trypanblue vital dye-exclusin) the hepatocytes were checked microscopically for morphological changes or reduction of the cell material.

Quantification of UDS: After coding 2 - 3 slides per test group were examined microscopically. At least 100 cells were evaluated per animal using at least 2 slides. By means of an automatic image analyzer the nuclear grain count (NGC) and the cytoplasmic grain count (CGC) was determined. The cytoplasmic area was adjacent to the nucleus and had approximately the same size of the nucleus.

The following parameters were calculated:

- the net nuclear grain count (NNGC = NGC - CGC)
- the mean nuclear grain count (NGC)
- the mean cytoplasmic grain count (CGC)
- the mean net nuclear grain count (NNGC)
- the percentage of cells in repair (cells with NNG ≥ 5)

4. Statistics:
In absence of an increased number of NNG no statistical evaluation was carried out.

5. Evaluation criteria:
A test substance is considered positive if an increase is demonstrated in both of the following:

- The mean net nuclear grain count (NNGC) must exceed zero at one of the dose groups
- The mean net nuclear grain count (NNGC) clearly exceeds the value of the concurrent negative control group at one of the dose groups

A dose-related increase between 5 and 20% of cells in repair (with an NNGC $\geq$ 5) outside the values of both the concurrent negative control and the historical control data base and a dose-related increase in the mean number of NNG counts near to but without exceeding zero is considered to be an indication for a marginal response which needs to be confirmed / clarified in a further experiment.

A test article producing both NNG counts and % cells in repair in all dose groups in the range of the negative control data is considered to be negative in the in vitro UDS assay.

II. RESULTS AND DISCUSSION

Due to unexpected death of six animals of the 1100 mg/kg bw dose group an additional test group with 275 mg/kg bw was performed and the remaining animals in the high dose group were killed without data generation. In addition, the preparation of the cultures of the vehicle control animals performed in parallel was discontinued.
In parallel to every dose group at least one vehicle control animal was prepared. Due to the use of animals from two separate shipments two animals from each shipment were prepared and evaluated for the vehicle control groups of both sampling times. Therefore, the sample of evaluation contained four animals for both vehicle control groups and only three animals for the test substance treated dose groups as recommended by OECD Guideline 486.

A. ANALYTICAL DETERMINATIONS

The stability of the test substance at room temperature in the vehicle deionized water over a period of 4 days was verified analytically. The homogeneity of the test substance in the vehicle was given by the fact that a solution was obtained. Additionally, homogeneity was assured by analytical determination of 3 individual samples of each concentration. The analytically determined mean concentrations were 27.866/27.835, 59.455/58.016 and 111.322/118.995 mg/mL at nominal concentrations of 27.5, 55.0 and 110.0 mg/mL, respectively. This corresponds to a recovery rate ranging between 101.2% to 108.2% and is thus, within the expected range (90-110%).

B. PRELIMINARY RANGE-FINDING TEST

In the pretest for the determination of the acute oral toxicity, deaths in males and females were observed down to a dose of 1360 mg/kg bw.. The clinical signs were piloerection, abdominal position, reduced general condition and shallow respiration. Therefore 1100 mg/kg was chosen as high dose for the main experiment.

C. CLINICAL EXAMINATIONS / MORTALITY

The single oral administration of the vehicle deionized water in a volume of 10 mL/kg bw was tolerated by all animals without any clinical observations.
The administration of the test substance did not lead to signs of toxicity at the dose groups 275 and 550 mg/kg bw. However, one animal treated with 550 mg/kg bw died within 3 hrs after administration. In contrary to the data of the pretest deaths were observed in the highest administered dose group of 1 100 mg/kg bw. At 3-hour sampling time two animals died and at 14-hour sampling time 4 animals died unexpectedly.
The single administration of the positive control substance 2-AAF in a dose of 50 mg/kg bw did not cause any evident signs of toxicity.

D. UDS ASSAY

No increase in the mean number of net nuclear grain counts was observed in any Reg.No. 88691 treated group, neither at the 3 hours nor at the 14 hours treatment interval [see Table 5.4/11]. The individual mean net nuclear grain counts of the test substance treated dose groups were between -1.65 and -7.94 and thereby close to the respective vehicle control values (-2.09 to -7.06) and nearby the historical negative control data range (-1.96 to -7.92; see data in report).

There was equally no relevant increase in the rate of cells in repair (NNGC ≥ 5). The rate of cells in repair per animal was in the range from 0% to 7%. The values were close to the range of the respective vehicle control values (0% to 5%) and close to the range of the historical negative control data (0% to 4%; see data in report).

The positive control 2-AAF, however, led to a marked increase of net nuclear grain counts and of the percentage of cells in repair at both treatment intervals, thus demonstrating the sensitivity and validity of the test.

Table 5.4/11 In-vivo UDS-test in Reg.No 88691 treated rats (Group Means ± SD)

Test groups	Vehicle control deionized water	275 mg/kg bw Reg.No. 88691	550 mg/kg b.w. Reg.No. 88691	Positive control 50 mg/kg bw 2-AAF
Sacrifice 3 hours after treatment				
NG counts	11.64 ± 4.87	15.44 ± 1.47	9.21 ± 0.30	21.68 ± 2.20
CG counts	16.12 ± 6.80	20.73 ± 5.99	12.23 ± 2.25	12.05 ± 3.07
NNG counts	-4.49 ± 2.30	-5.30 ± 2.31	-3.02 ± 2.08	9.64 ± 3.56
% cells in repair NNG ≥ 5	2	3	4	69
Sacrifice 14 hours after treatment				
NG counts	9.73 ± 3.67	12.67 ± 1.98	5.90 ± 1.87	22.72 ± 2.06
CG counts	14.76 ± 5.38	17.62 ± 2.26	8.08 ± 1.82	11.71 ± 1.24
NNG counts	-5.03 ± 2.08	-4.96 ± 0.66	-2.18 ± 0.16	11.00 ± 4.47
% cells in repair NNG ≥ 5	2	1	2	76

NG: Mean nuclear grain; CG: Mean cytoplasmic grain; NNG: Mean net grain;

E. CYTOTOXICITY

The cell viability of the individual hepatocytes preparations varied between 70.4% and 90.5%.The data concerning the cell viability show that the cell preparation was adequately and the test substance treatment did not influence this quality parameter, because the mean relative viabilities ranged between 100.0 to 102.3% of the mean control value for the hepatocytes isolated 3 hours after administration and of 91.4 to 104.6% for the hepatocytes isolated after 14 hours. No changes in cell morphology after test substance treatment were observed.

F. COMMENT TO STUDY CONDUCT

Due to the unexpected deaths observed in the 1st Experiment at the previously defined maximum tolerated dose of 1 100 mg/kg body weight a 2nd Experiment with an additional dose group (275 mg/kg bw.) at both sacrifice intervals was performed to fulfill the requirements of OECD Guideline 486. It was assumed that the reason of the unexpected lethality in the 1st Experiment that the animals of the pretest were not starved before and after the test substance administration.

In both experiments animals from different shipments were used. Therefore, for the vehicle control group of both sampling times two animals each were evaluated. No relevant differences in DNA repair activity were obtained. Thus, these changes had no detrimental impact on the validity of this study.

After single oral administration of the vehicle deionized water the mean number of net nuclear grain counts (NNGC) in these vehicle control animals was within the range of the historical vehicle control data (see data in report). In addition, the number of cells in repair was in the expected range.

III. CONCLUSIONS

Under the experimental conditions of this in vivo study, the test substance Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) does not induce unscheduled DNA synthesis in rat hepatocytes isolated 3 or 14 hours after oral administration of a dose of 275 or 550 mg/kg bw.

5.4.6 In vivo studies in germ cells

The very extensive data base on genotoxicity of Bentazone-sodium did not give rise to any concern. Therefore, an additional genotoxicity study in germ cells is not required. The absence of this study does not constitute a data gap.

Literature - Wing spot test in Drosophila melanogaster

Report:	II A 5.4/1 Kaya B. et al. 2003(a) Evaluation of the genotoxicity of four herbicides in the wing spot test of Drosophila melanogaster using two different strains BASF DocID 2011/1257369
Date of report:	27-Sep-2003
Testing facility:	not applicable
Guidelines:	not applicable
GLP:	No, not subject to GLP regulations

Executive Summary of the Literature

Bentazone (purity > 95%, obtained from Koruma Tarim Ilaclari, Istanbul, Turkey) together with three other pesticides was evaluated for mutagenic and recombinagenic effects using the wing spot test of Drosophila melanogaster (somatic mutation and recombination test, SMART). Both standard and high-bioactivation fly crosses were used, the latter cross is characterised by a high cytochrome P-450-dependent bioactivation capacity.
Three-day-old larvae, transheterozygous for the multiple wing hairs and flare-3 genes, were chronically fed with six different concentrations of bentazone dissolved in 5% acetone. Feeding ended with pupation of the surviving larvae and the genetic changes induced in somatic cells of the wing's imaginal discs lead to the formation of mutant clones on the wing blade. Point mutation, chromosome breakage and mitotic recombination are known to produce single spots; while twin spots are produced only by mitotic recombination. Ethylmethansulfonate was used as positive control.

Bentazone gave a negative response in the standard cross but a positive result in the wing spot test with the high-bioactivation cross. All concentrations tested (0.05 mM – 5 mM) showed a significantly increased frequency of total spots, mainly due to induction of small single spots in the marker-heterozygous wings. This increase was without a clear dose-response relation and is discussed as induced by a metabolite formed in vivo in this invertebrate or a degradation product of bentazone.

Despite the fact that this test is an in vivo genotoxicity test, it is considered as screening test at first level and does not have regulatory relevance. Furthermore, it is mentioned that the frequency of total spots in the negative controls in this study is smaller than expected according to other reported data. However, this study indicates some genotoxic activity of a metabolite or degradation product formed in the high-bioactivation insect Drosophila melanogaster.
This data is regarded as supplementary information without regulatory relevance. It does not have an impact on the risk assessment.

5.5 Long-term toxicity and carcinogenicity

Studies presented in the original Annex II Dossier (1995): Bentazone-acid was tested for chronic toxicity and potential carcinogenicity in rats and mice. **Bentazone-sodium** was not tested. The studies conducted are well in line with the OECD guideline-requirements of that time. The studies have been evaluated by European authorities and Germany as Rapporteur member state in 1998 (European Commission Peer Review Program, BASF Doc ID 1998/1001178) and were considered to be acceptable. For the convenience of the reviewer, these studies are listed in Table 5.5/1.

Based on these long-term studies in rats and mice a carcinogenic potential of Bentazone was excluded. In both species, an impairment of blood coagulation and minor effects on liver and kidney were observed. In the most current chronic/oncogenicity study in mice blood coagulation was impaired at 400 ppm (47 mg/kg bw). In addition at 2,000 ppm the incidence of hemorrhage in liver and heart was increased. In rats, blood coagulation effects were seen at 800 ppm (47 mg/kg bw) in males and one male died at 4,000 ppm due to hemorrhagic lesions. Frequently, a reduced body weight (gain) and diminished food consumption were noted at higher dose levels. The toxicological significance of effects on the eyes observed in one rat study and of an increased testicular calcification in male mice is equivocal. These effects were evaluated to be not likely to be treatment-induced.
The lowest NOEL for chronic toxicity of about 10 mg/kg bw/d was derived from a study in Fischer 344 rats. In the most recent chronic feeding study in mice, the NOEL was about 12 mg/kg bw/d.
As indicated by the NOELs determined in subchronic and long-term studies in rats, mice and dogs, interspecies variation of Bentazone toxicity seems to be rather low.

Table 5.5/1 Summary of peer-reviewed long-term toxicity studies with Bentazone

Study Dose levels (Batch / purity)	Sex	NOAEL ppm (mg/kg bw/d)	LOAEL ppm (mg/kg bw/d)	Effects at LOAEL and above	Reference (BASF DocID)
Rat (Sprague-Dawley), 2-year (oral) chronic toxicity, diet M & F: 0-100-350-1,600 ppm (batch & purity not given)	M & F	350 (17)	1,600 (76)	Reduced body weight and food consumption; increased organ weights	[redacted] 1974(a) Doc ID 1974/004
Rat (Fischer 344, Du/Crj (SPF)), 2-year (oral) combined chronic toxicity / carcinogenicity, diet M & F: 0-200-800-4,000 ppm (N 169; 93.9%)	M F	200 (9) 200 (11)	800 (35) 800 (45)	Impaired blood coagulation, impaired liver and kidney function indicated by changes in clinical chemistry and organ weights. At 4,000 ppm reduced body weight	[redacted] 1984 (a) Doc ID 1985/433 [redacted] 1985(a) Doc ID 1985/440 B[redacted] 1986(a) Doc ID 1986/0438 [redacted] 1989(a) Doc ID 1989/10485 [redacted] 1988 (a) Doc ID 1988/0155
Mice (Swiss Webster) 18 month oral carcinogenicity study, diet M & F: 0-100-350-1,600 ppm (batch & purity not given)	M & F	350 (52)	1,600 (237)	High mortality (>50%) in test and control groups. At 1,600 ppm reduced food consumption and body weight, organ weight changes.	[redacted] 1974(a) Doc ID 1974/041
Mice (CFLP) 82-95 week oral carcinogenicity, diet M & F: 0-100-350-1,600 ppm (p.195.75 / purity not given)	M F	1600 (138.4) 1600 (152.8)	– –	No adverse effects	[redacted] 1978(a) Doc ID 1978/034
Mouse (B6C3F1) 2-year (oral) combined chronic toxicity/carcinogenicity, diet M & F: 0-100-400-2,000 ppm (N 169; 93.9%)	M & F	100 (12)	400 (47)	Impaired blood coagulation, increased testicular calcification (questionable effect, not confirmed in other studies); proliferative lesions in the liver (females) At 2,000 ppm transient reduction of body weight gain (males), hemorrhages in liver and heart	[redacted] 1984 (b) Doc ID 1985/432 [redacted] 1985 (a) Doc ID 1985/431 [redacted] 1987(a) Doc ID 1987/0139 [redacted] 1988 (a) Doc ID 1988/0483 [redacted] 1987(a) Doc ID 1987/10417

Regarding all information available from the chronic studies, the overall conclusion can be drawn that bentazone has no carcinogenic potential although the compound may have caused proliferative lesions in the liver of female mice.

Submission of not yet peer-reviewed studies in this AIRII-Dossier:
No new long-term toxicity studies with Bentazone were considered to be necessary for submission within the AIR II process.

In summary, a classification for carcinogenicity is considered to be not justified for Bentazone on the basis of the long-term studies performed with Bentazone-acid in accordance to GHS-classification criteria laid down in the Council Regulation 1272/2008/EC and OECD Globally Harmonized System (GHS, 2009). Under consideration of the demonstrated equivalence of the acid (BAS 351 H) and the sodium salt (BAS 351 H-Na) derivative after oral administration, this classification proposal is also considered justified for the manufactured use product Bentazone-sodium.
The agreed endpoint for derivation of the Acceptable daily intake (ADI) was based on the more recent combined chronic toxicity / carcinogenicity study in rats ([redacted] 1985) with a NOAEL of 200 ppm, equivalent to 10 mg/kg bw (combined sexes). This approach is still valid.

Therefore, the endpoints fixed in the European Commission Review report for the active substance bentazone (7585/VI/97-final from 30 November 2000) regarding long-term toxicity, carcinogenicity and ADI Reference value are considered to be still valid and might be supplemented with regard to Bentazone-sodium.

EU agreed Endpoints for bentazone (EC Review report, 2000)

Long term toxicity and carcinogenicity

Target / critical effect:	Impairment of blood coagulation; effects on liver and kidney
Lowest relevant oral NOAEL / NOEL:	200 ppm (10 mg/kg bw/d), 24-month rat study
Carcinogenicity:	No carcinogenic potential

ADI derivation

ADI	0.1 mg/kg bw/d (based on 24-month rat feeding study and a safety factor of x100)

5.5.1 Long-term (2 years) oral toxicity in the rat

No new studies were performed. All information available is presented in the original Annex II Dossier (1995) and has been evaluated by European authorities and Germany as Rapporteur member state.

5.5.2 Carcinogenicity study in the rat

No new studies were performed. All information available is presented in the original Annex II Dossier (1995) and has been evaluated by European authorities and Germany as Rapporteur member state.

5.5.3 Carcinogenicity study in the mouse

No new studies were performed. All information available is presented in the original Annex II Dossier (1995) and has been evaluated by European authorities and Germany as Rapporteur member state.

5.5.4 Mechanism of action and supporting data

No data, not required.

5.6 Reproductive toxicity

Since the last EU peer-review, additional information on reproductive toxicity of Bentazone has become available which is considered in this updated Dossier. The results of these published investigations however do not change the previous evaluation of Bentazone.

Studies presented in the original Annex II Dossier (1995): Bentazone-acid was investigated in two multi-generation studies in rats and in several prenatal toxicity studies in rats and rabbits. The studies revealed neither teratogenic potential nor impairment of the reproductive performance. **Bentazone-sodium** was not tested for reproductive toxicity by BASF, but developmental toxicity studies were submitted by Agrichem and were evaluated in the Monograph (Vol.3, Annex B-5, September 1996, chapter 5.6.2.1) indicating equivalence to the acid form. The studies conducted are well in line with the OECD guideline-requirements of that time and most are conducted according to GLP. The studies have been evaluated by European authorities and Germany as Rapporteur member state in 1998 (European Commission Peer Review Program, BASF Doc ID 1998/1001178) and were considered to be acceptable. For the convenience of the reviewer, all studies submitted within the first Annex I inclusion process are listed in Table 5.6/1.

The multi-generation studies (one 3-generation and one 2-generation study) did not show any adverse effects on reproduction or any evidence of teratogenicity of bentazone. The lowest relevant NOEL was 200 ppm (about 14 mg/kg bw) based on slightly reduced pup body weights. The NOEL for systemic parental toxicity was set at 800 ppm (about 56 mg/kg bw) based on slightly reduced body weight in the parental animals at the highest dose level (3200 ppm).

The lowest NOEL in developmental toxicity studies in rats was 100 mg/kg bw, both for maternal and fetal effects. Maternal effects under the test conditions were restricted to slightly decreased food consumption in the highest dose of 250 mg/kg bw. Fetal effects were manifested as reduced fetal weights, increased late resorptions as well as incomplete ossification. For bentazone, reduced food consumption was shown in subacute and subchronic studies to be a relatively late indicator for toxicity. It is very likely that maternal toxicity under consideration of the typical target organs (blood, kidney, liver) was present, which however was not investigated in this study. This conclusion is also supported by a prenatal *dietary* study in rats with substance administration from day 0 to day 21 of gestation. The maternal NOEL was 2,000 ppm (equal to 162 mg/kg per day) based on significantly higher water consumption and increased amniotic fluid at 4,000 ppm (equivalent to 324 mg/kg bw). Severe maternal effects like decreased weight gain and decreased food consumption and signs of hemorrhagic diathesis were seen at 8,000 ppm (equivalent to 631 mg/kg bw). The NOEL for embryo-/fetotoxicity was 4,000 ppm (324 mg/kg bw) based on reduced fetal body weight and retarded ossification in the top dose level. There was no difference regarding postimplantation loss documented in any dose group. Although this study was considered only as supplementary, it provides important additional information for the most relevant exposure route for assessment of consumer safety.

The developmental toxicity studies in rabbits revealed a maternal and developmental NOEL of 150 mg/kg bw. Maternal toxicity was based on reduced food consumption in the high dose group at 375 mg/kg bw. Developmental toxicity manifested as increased postimplantation losses and one complete litter loss. Bentazone was not teratogenic in rabbits.

Table 5.6/1 Summary of peer-reviewed reproduction toxicity studies with Bentazone

Study Dose levels (Batch / purity)	Endpoint	NOAEL ppm (mg/kg bw/d)	LOAEL ppm (mg/kg bw/d)	Effects	Reference DocID
Multi-generation studies					
Rat (Sprague-Dawley), Multigeneration (3-gen) study, **diet** 0-20-60-180 ppm (batch / purity not given)	Fertility	180 (18, hdt)	–	No evidence of impaired fertility	[redacted] 1973 Doc ID 1973/010
	Offspring toxicity	180 (18, hdt)	–	No offspring toxicity	
	Parental toxicity	180 (18, hdt)	–	No parental toxicity	
Rat (Wistar/HAN), Multigeneration (2-gen) study, **diet** 0-200-800-3,200 ppm (N187 / 97.8%)	Offspring toxicity	200 (14)*	800 (56)*	Reduced pup body weight secondary to maternal toxicity	[redacted] 1989 * Doc ID 1989/0068
	Parental toxicity	800* (56)*	3,200* (247)*	Reduced food consumption and body weight of F0 dams at PND 1-4	
Developmental studies in rats					
Rat (Sprague-Dawley) Prenatal developmental toxicity, **oral gavage** during days 6-15 of gestation 0-22.2-66.7-200 mg/kg bw/d (batch/purity not available)	Development	- (66.7)	- (200)	Elevated resorption rate, reduced bw, evidence of teratogenicity (increased number of runts, anasarca) *Remark: Teratogenic effects not reproducible* (see 1978/039)	[redacted] (1971) Doc ID 1971/0041
	Maternal tox.	- (200, hdt)	-	No adverse effects	
Rat (Sprague-Dawley) Prenatal developmental toxicity, **oral gavage** during days 6-15 of gestation 0-22.2-66.7-200 mg/kg bw/d (batch not available/ 92.5%)	Development	- (200, hdt)	-	No adverse effects	[redacted] 1978) Doc ID 1978/039
	Maternal tox.	- (200, hdt)	-	No adverse effects	
Rat (Wistar) Prenatal developmental toxicity, **oral gavage** during days 6-15 of gestation 0-40-100-250 mg/kg bw/d (N 187/ 97.8%)	Development	(100)	- (250)-	Increased fetal resorptions and retarded fetal development. Not teratogenic	[redacted] 1986 Doc ID 1986/421
	Maternal tox	- (100)	- (250)	Reduced food consumption	

Rat (SD:CRJ) Prenatal developmental toxicity, **diet during** days 0-21 of gestation 0-2,000-4,000-8,000 ppm 0-162-324-631 mg/kg bw/d (N169 / 93.9%)	Development	4,000 (324)	8,000 (631)	Reduced body weight and ossification of cervical vertebrae Not teratogenic	[redacted] 1982 Doc ID 1984/066
	Maternal tox.	2,000 (162)	4,000	Increased water consumption and amniotic fluid 8,000 ppm: reduced bw gain, hematuria	
Published literature: Rat (strain not given) Prenatal developmental toxicity, **single oral gavage** on day 6, 8, 11, 14 or 16 of gestation 0-25 - 90- 200 mg/kg bw **Basagran** corresponding to 0- 12- 43.2-96 mg/kg bw bentazone (batch / purity not submitted)	Development	- (200, hdt)	–	Increased resorption rate, retardation of fetal development and incomplete ossification in all treated groups. No dose-response relationship. Inconsistency of data. No assessment possible.	[redacted] 1988 Doc ID 1988/10538
	Maternal tox.	No data given			
Developmental studies in rabbits					
Rabbit (Himalayan, ChBB:HM) Prenatal developmental toxicity, **oral gavage** during days 6-18 of gestation; 0-50-100-150 mg/kg bw/d (batch not available / 92.5%)	Development	- (150, hdt)	-	No adverse effects	[redacted] Doc ID 1984/048
	Maternal tox.	- (150, hdt)	-	No adverse effects	[redacted] 1984(a) Doc ID 1984/288
Rabbit (Chinchilla) prenatal developmental toxicity, **oral gavage** during days 6-18 of gestation 0-75-150-375 mg/kg bw/d (N187 / 97.8%)	Development	- (150)	- (375)	Total post implantation loss in one dam. Not teratogenic	[redacted] 1987 Doc ID 1987/058
	Maternal tox.	- (150)	- (375)	Reduced food consumption	

* Study has been re-evaluated under AIR2 Dossier Chapter 5.6.1/1 and new endpoints are considered justified.

Taken all information together, the prenatal studies in rats and rabbits and the multigeneration studies in rats did not reveal a teratogenic potential or any adverse effects of bentazone on the reproductive performance (see Monograph on Bentazone, see Vol.1, Level 2, chapter 2.4.1). According to the European Review Report for Bentazone the critical effect regarding reproduction is reduced pup weight at parental toxic doses.

Submission of not yet peer-reviewed studies in this AIR2-Dossier:
No new studies on reproductive toxicity with Bentazone were considered to be necessary for submission within the AIR2 process.
In view of the most current evaluation of the Bentazone data package by EPA [Bentazone Registration review: Revised Human-Health Assessment Scoping Document; March 2010] which concludes that bentazone impairs offspring development at non parentally toxic doses, a re-evaluation of the 2-generation study has been performed and is presented in this dossier (see AIR2 Dossier II A 5.6.1/1). This re-evaluation clearly advocates the European conclusion that pup weight effects are secondary to maternal toxicity (presented in the Final Review Report on bentazone, see EC 7585/VI/97, 30 November 2000) as it was possible to show that reduced pup weight gain was predominantly seen in dams with markedly reduced food consumption. Furthermore, the effect levels were recalculated based on the actual food intake in the sensitive period (PND1-4), which is higher than the average food intake during gestation.

Based on this re-evaluation, the no observable adverse effect level (NOAEL) is still 200 ppm, but is valid for both developmental and maternal effects. The maternal lowest observable adverse effect level (LOAEL) is set to 800 ppm based on reduced food consumption and body weight gain during post natal days 1-4 in individual dams. This reduced maternal nutritional intake is shown to subsequently influence the pup body weight gain of the respective dams. According to the substance intake during the relevant time interval post natal day 1-4, the NOAEL is equivalent to 22 mg/kg bw and the LOAEL is set at 80 mg/kg bw. The new no observed effect levels will not have an impact on the human health risk assessment.

Furthermore several publications were found in the external literature search (for search profile see Literature Search Report Doc ID 2012/1007281, for summary of results see Excel file Doc ID 2012/1007283) which report investigations of Bentazone's endocrine activity. The publications are reviewed in detail under point 5.6.6/1and 5.6.9/1-5, whereas here a short summary is given:
No adverse effect was found in an in vivo subchronic drinking water study on spermatogenesis in CD1 mice . No binding affinity or transcriptional activity for the human estrogen receptor was found in yeast or mammalian cells (CHO). In addition, in MCF-7 cells Bentazone did not show agonistic activity towards the estrogen receptor. An anti-androgenic transcriptional activity was reported for high concentrations in yeast cells. In mammalian cells (CHO) no transcriptional activity was reported. Furthermore, in an immuno-immobilized human androgen-receptor binding assay no binding ability of Bentazone was found up to the highest soluble concentration. Furthermore, Bentazone showed no effect on steroidogenesis in the Ovulation screening assay in Xenopus oocytes.
Taken all together, Bentazone was shown to have no endocrine activity in several screening studies. The results of these investigations are in line with the available higher tier studies in rats and rabbits, which also did not provide evidence for an endocrine potential of Bentazone.

The data submitted in this chapter are summarized in Table 5.6/2.

Table 5.6/2 Summary of reproduction toxicity studies with BAS 351 H

Study Dose levels (Batch / purity)	Endpoint	NOAEL ppm (mg/kg bw/d)	LOAEL ppm (mg/kg bw/d)	Effects	Reference BASF DocID
Rat (Wistar/HAN), Multigeneration (2-gen) study*, diet 0-200-800-3,200 ppm (N187 / 97.8%)	Fertility* changed in comparison to DAR (1996)	3,200* (230)* Not derived*	–	No evidence of impaired fertility	AIR2-Dossier II A 5.6.1/1 [redacted], 2011 Doc ID 2011/1248852 AIR2-Dossier II A 5.6.1/2 [redacted] 2011 Doc ID 2011/1145234 AIR2-Dossier II A 5.6.1/3 [redacted], 2009 Doc ID 2011/1262290
	Offspring toxicity* changed in comparison to DAR (1996)	200 **(22)*** 200 (14)*	800 **(80)*** 800 (56)*	Reduced pup body weight secondary to maternal toxicity	
	Parental toxicity* changed in comparison to DAR (1996)	**200*** **(22)*** 800* (56)*	**800*** **(80)*** 3,200* (247)*	Reduced food consumption and body weight of F0 dams at PND 1-4	
Literature: Mice (CD1) Subchronic (100 days) vs. pre natal (complete gestation) & subchronic adminstration via drinking water 30 µg/l	Development	30 µg/l (hdt)		No adverse effects	AIR2-Dossier II A 5.6.6/1 Garagna S. et al. 2005 Doc ID 2011/1257366
	Parental tox.	30 µg/l (hdt)		No adverse effects	
Literature: Human Estrogen /Androgen receptor transactivation-Assay / Yeast-cells 0.01-1000 µM Ovulation Assay in cultured Xenopus oocytes 6.25-62.5 µM (batch not reported/ >97%)	Antiestrogenic/ Estrogenic activity Antiandrogenic / Androgenic activity Ovulatory response	- < 500 µM / - -	- ≥500 µM / - -	No transcriptional activity Antiandrogenic transcriptional activity at high concentrations No effect on steroidogenesis	AIR2-Dossier II A 5.6.9/1 Orton F. et al. 2009(a) Doc ID 2011/1257367
Meta-Literature: Human Estrogen receptor □ binding ability and transcriptional activity (tested concentration, batch and purity not reported)	hER□□ binding ability hER□□ transcriptional activity	- -	- -	No binding ability No transcriptional activity	AIR2-Dossier II A 5.6.9/2 Roncaglioni A. et al. 2008(a) Doc ID 2011/1257368
Literature: Human Estrogen (□□□□ /Androgen receptor transactivation-Assay /CHO-cells Tested up to 10 µM (batch not reported/ >95%)	Antiestrogenic/ Estrogenic activity Antiandrogenic / Androgenic activity	- -	- -	No transcriptional activity No transcriptional activity	AIR2-Dossier II A 5.6.9/3 Kojima H. et al. 2004(a) Doc ID 2004/1036097

Table 5.6/2 Summary of reproduction toxicity studies with BAS 351 H

Study Dose levels (Batch / purity)	Endpoint	NOAEL ppm (mg/kg bw/d)	LOAEL ppm (mg/kg bw/d)	Effects	Reference BASF DocID
Literature: Immuno-immobilized human Androgen receptor binding assay Tested up to highest soluble concentrate (batch not reported/ 99.9%)	rh AR binding ability	-	-	No binding ability	AIR2-Dossier II A 5.6.9/4 Bauer E.R.S. et al. 2002(a) Doc ID 2011/1257371
Literature: E-Screen-Assay / MCF-7 cells 0.01-1000 µM (batch not reported/ 99.9%)	Estrogen-receptor mediated proliferation	-	-	No estrogen agonistic activity	AIR2-Dossier II A 5.6.9/5 Bitsch N. Et al., 2002 Doc ID 2011/1281908

hdt: highest dose tested

* 2-gen-Study DocID 1989/0068 has been re-evaluated. Both new and former effect levels are presented.

A classification for reproductive toxicity is not considered to be required for Bentazone (BAS 351 H) or for the manufactured use product Bentazone-sodium (BAS 351 H-Na) in accordance to GHS-classification criteria laid down in the Council Regulation 1272/2008/EC and OECD Globally Harmonized System of Classification and Labelling of Chemicals (GHS, 2009).
This is based on the following reasoning:
Bentazone was shown to be not teratogenic and to have no impact on the reproductive performance. Developmental changes consistently found in gavage and dietary studies were small reductions in fetal or pup body weight accompanied with retarded ossification at maternally toxic dose levels. These minor developmental effects are considered to be not relevant for classification. Significant effects (fetal resorptions) were restricted to developmental studies with bolus administration via gavage and were not reproducible in dietary studies, despite considerably higher dose levels (8000 ppm or about 630 mg/kg/day, which are close to the dose which resulted in mortality after short term administration). Therefore fetal resorptions are considered to be peak plasma effects which are based on the rapid substance uptake and the high plasma concentrations achieved after gavage. As dietary studies did not lead to fetal resorptions up to the highest dose levels it can be concluded that significant effects would not occur under realistic exposure scenarios.

The endpoints which were fixed in the European Commission Review report for the active substance bentazone (7585/VI/97-final from 30 November 2000) for reproduction are therefore considered to be still valid with minor changes concerning the substance intake. However, the changes have no impact on the overall human health risk assessment.

Proposal for Endpoints for Bentazone

Reproductive toxicity (changes to the EU agreed endpoints of the EC Review report (2000) are indicated in bold)

Target / critical effect - Reproduction:	reduced pup weight at parental toxic doses
Lowest relevant reproductive NOAEL / NOEL:	200 ppm (**22 mg/kg bw/d**), 2-generation rat study
Target / critical effect- Developmental toxicity:	No teratogenic potential
Lowest relevant developmental NOAEL / NOEL:	100 mg/kg bw/d (rat)

5.6.1 Two generation reproductive toxicity in the rat

Report: II A 5.6.1/1
[redacted] 2011(a)
Bentazone (BAS 351 H): Reassessment of the maternal and developmental toxicity in the 2-generation study
BASF DocID 2011/1248852
Date of report: 03-Sep-2011
Testing facility: [redacted].
Guidelines: not applicable
GLP: No, not subject to GLP regulations
Note: The original two-generation study (see Monograph, Vol.3, Annex B-5, champter 5.6.1.2, BASF DocID 1989/0068) is re-evaluated in due consideration of historical control data (DocID 2011/1145234) and including a focus on cofactors like individual animal food consumption and animal weight data as well as litter size distribution in order to decide whether the pup weight impairment is a primary substance effect of bentazone or a secondary due to impairment of the dams.

References within the Evaluation (DocID 2011/1248852):

Report: II A 5.6.1/2
[redacted]2011(a)
8 Two-generation studies 1985-1990 in the Han Wistar rat (KFM:WIST) performed at RCC Ltd.
BASF DocID 2011/1145234
Date of report: 29-Aug-2011
Testing facility: [redacted]
Guidelines: not applicable
GLP: No, not subject to GLP regulations
Note: Historical control data report

Report: II A 5.6.1/3
Chahoud I., Paumgartten F.J.R. 2009(a)
Influence of litter size on the postnatal growth of rat pups: Is there a rationale for litter-size standardization in toxicity studies?
BASF DocID 2011/1262290
Date of report: 16-Sep-2009
Testing facility: not applicable
Guidelines: not applicable
GLP: No, not subject to GLP regulations
Note: Literature: Alternative procedures to attenuating litter-size-induced variability in pup growth

Executive Summary

The herbicide Bentazone, BAS 351 H, was positively evaluated for Annex I inclusion based on the dossier submitted in 1995 by BASF AG. With regard to developmental endpoints Bentazone has been evaluated in a two-generation study [1989; Doc ID 1989/0068]. This study showed slightly reduced pup weight effects in the F1 and F2 generation only at the mid dose (800 ppm) in the absence of obvious effects on parental weights; whereas at the high dose (3200 ppm) parental mean weights were in addition significantly reduced. However, data from the chronic rat study showed significant haematological and clinical chemistry alterations at 800 ppm.
Although the European Commission Final Review Report on bentazone [see EC 7585/VI/97, 30 November 2000] considered the effects demonstrated in the chronic study for the evaluation of the parental toxicity, in the most current evaluation of the same data by EPA [Bentazone Registration review: Revised Human-Health Assessment Scoping Document; March 2010] this was not considered. Thus, the EPA considers bentazone to effect the offspring at non parentally toxic doses.

In order to re-assess these conflicting evaluations and to get a better picture about the origin of the pup weight effects, the two-generation study was re-evaluated with a focus on:

- historical control data on mean maternal and mean pup weight
- litter size distribution and
- maternal toxicity

The reduced mean pup weight in the mid dose F1-generation was shown to be attributed to a considerable extent to the small litter size of the control group, since normalization of the mean pup weight to the litter size reduced this difference from maximal 12 to 7%. In a next step the remaining pup weight deviations were shown to be associated with litters from F0 dams showing a significantly reduced food intake and reduced body weight gain in the relevant period of the lactation phase (PND 1-4).
Similarly, the small mean litter size of the control animals in the F2 generation led to higher mean pup weights in the control litters as compared to the treated groups. This led to an artificially high deviation of the treated animals to the control, but actually the F2-generation mean pup weights are well within the naturally occurring variability of this parameter.

Therefore, this evaluation definitely supports the European conclusion that pup weight effects were observed only at maternally toxic levels. This re-evaluation shows the maternally toxic dose to lie at 800 ppm in the F0 parent generation. The calculation of the effect levels in mg/kg bw was done under consideration of the actual intake during the period where the toxicity was observed, namely during the lactation period PND 1-4. The following effect level is therefore considered reasonable:

	NOEL	LOEL	Relevant effect
Parental & developmental toxicity	200 ppm [~22 mg/kg bw]	800 ppm [~80 mg/kg bw]	Reduced parental food consumption and body weight gain Reduced pup body weight resulting from parental toxicity

Introduction

The active substance BAS 351 H, Bentazone-acid, is an herbicide that was positively evaluated for Annex I inclusion based on the dossier submitted in 1995 by BASF AG.
The dossier contained 2 two-generation studies [Leuschner F. et al., 1973; BASF Doc ID 1973/010 and Suter P. et al., 1989; Doc ID 1989/0068]; the first dosed below the NOAEL and the last dosed at higher levels. This latter one is object of discussion herein. The Rapporteur member state Germany evaluated this study as acceptable and concluded that reproductive/developmental toxicity, based on slightly reduced body weight development of pups in the F1 and F2 generation was seen at 800 ppm, while obvious systemic parental toxicity was found at 3200 ppm based on general body weight reduction.
Overall, the reproductive/developmental toxicity in the offspring was commented in the DAR as being well in line with the effect/no observed effect levels in the chronic rat studies, in which impairment of blood coagulation as well as liver and kidney function were noted at 800 ppm by changes in clinical chemistry and urinalysis parameters, but effects on body weight were initially seen at higher doses. Based on the same data, EPA suggested that Bentazone seems to have a higher susceptibility towards the offspring after in utero and postnatal exposure.

In this assessment, the two-generation study is re-evaluated in due consideration of historical control data and including a focus on cofactors including individual animal food consumption and animal weight data as well as litter size distribution in order to decide whether the pup weight impairment is a primary substance effect of bentazone or a secondary due to impairment of the dams' health.

Description of the 2-generation study as given in the report

Two multi-generation studies were conducted with BAS 351 H in rats. The first one was a 3-generation study (Doc ID 1973/010) in Sprague-Dawley rats which revealed no effect up to the highest dose tested (180 ppm equivalent to 21.9 mg/kg bw/day). This study is not considered furthermore in this assessment.

The second one was a 2-generation study (Doc ID 1989/0068) in Wistar rats performed at [redacted] under project number 67757 in 1986 to 1987. Bentazone was administered to groups of 25 male and 25 female young rats (F0 parental generation) as a constant homogeneous addition to the diet in concentrations of 0, 200, 800, or 3200 ppm (corresponding to a mean intake of around 0, 14, 56 or 230 mg/kg bw/day during pre-pairing and gestation period). After a pretreatment period of 70 days the F0 animals were mated to produce the F1 litter and the dams reared their offspring until post natal day (PND) 4 and, after standardization to 8 pups, proceeded until day 21 after parturition.

After weaning 25 males and 25 females were selected from these F1 pups as the F1 parental generation and were offered the same dietary doses as their parents for 123 days before they were paired to produce a second litter (F2 pups). Like the F0 females, the F1 females were allowed to litter and rear their F2 pups until post natal day 4 and, after standardization, until day 21 after parturition.

The overall results were summarized in the two-generation study report [1989/0068] as follows:

(i) **Parental animals of F0 and F1 generation**: Decreased mean body weights in the high dose (3200 ppm) F1 generation males during the prepairing and post-pairing periods and in the F0 and F1 generation females during all periods. Significance was given, as described in the statistical report (p. 46 of the original study report), at days 1-4 of the lactation period in F0 females and at isolated time points during all periods in the F1 females.

(ii) **Pup development**: Body weight development was impaired in the F1 and F2 pups in the mid (800 ppm) and high dose (3200 ppm). Significance was given in the F1 pups at 800 and 3200 ppm at day 1-21, in the 800 ppm F2 pups at day 4, 14 and 21 and in the high dose group from day 4 to 21.

(iii) **Not influenced in the 2-generation study** were the following parameters: Mortality, food consumption of P and F1 animals and food consumption of pup generations, viability of pups, general appearance, mating performance and fertility, duration of gestation, fertility index, postimplantation loss, parturition, quantity and quality of progeny, sex ratio of pups, postnatal loss up to day 4 post partum and breeding loss from day 4 up to day 21 post partum, lactation and nursing, development and behaviour of pups, incidence of malformations and/or anomalies, necropsy findings and histopathological findings.

(iv) **No teratogenic effect** of the test article was evident.

In this evaluation the effect of litter size differences and the normal range of variability were not further considered. The final conclusion was made based on mean substance intake data during gestation and without consideration of the actual substance intake during the relevant time frame of lactation. The derived effect levels as given in the Draft assessment report / Monograph are summarized in Table 5.6/3.

Table 5.6/3 Endpoints of the 2-generation study as stated in the Draft Assessment Report

	NOEL ppm [mg/kg bw]	LOEL ppm [mg/kg bw]	Relevant effect
Parental toxicity	800 [~56]	3200 [~230]	Parental body weight
Developmental toxicity	200 [~14]	800 [~56]	Fetal body weight

According to the conclusion drawn in the DAR, offspring toxicity was manifested as impaired pup development during lactation at a dose lower than the parental systemic toxicity which is, after comprehensive reassessment of the data as discussed in the following chapters, not the case.

Points that initiated the re-examination of specific data

Missing of historical control data
Historical control data are valuable to differentiate between effects observed in a study to a concurrent control group and inherent variability of biological parameters in studies conducted according to the same protocol in the same laboratory. As no historical control data were reported in the 2-generation reproduction study with BAS 351 H, those data were summarized in a separate report [redacted] Doc ID 2011/1145234) and are used to interpret the data in the context of the variability of pup weight development.

Check for Maternal toxicity
The mean food consumption of the F0 and F1 dams is stated to be similar in all groups. In contrast to this the mean body weight of the high dose F0 females is significantly reduced directly after parturition. To elucidate the reason for body weight effects the food consumption was rechecked in this time frame.

Littersize effect
The pup weight development in the F1 generation at 800 ppm is reduced at day 4 and 7. It was investigated whether the litter size pattern as shown in Table 5.6/4 attributes on the one side to the reduced mean pup weight and on the other side covers maternal effects on food consumption.

Table 5.6/4 Litter size pattern in the F1 generation

Litter size	Number of Litters with specific Litter size			
	Control	200 ppm	800 ppm	3200 ppm
<=8	4	2	1	2
9	2	1	1	1
10	2	3	2	5
11	6	3	5	4
12	4	8	4	3
13	3	6	4	5
>=14	1	2	5	3

Calculation of the effective dose based on substance intake during the effect period
As the effects occur in the lactation phase during which the food intake is higher than during gestation or pre-pairing period, it is reasonable to establish effect levels on a mg/kg bw level based on the actual substance intake within the respective period.

Historical control data

The historical control (HC) data for this 2-generation reproduction study with BAS 351 H are compiled in a separate report [redacted], Doc ID 2011/1145234) and are based on eight 2-generation studies, all conducted in the same strain of rats as the rats used in the BAS 351 H study. The studies were performed at RCC Ltd. during the years 1985 to 1989 as dietary studies with a pre-pairing period of 56 to 70 days in the P generation and of at least 101 days in the F1 generation. Therefore, they are well comparable to the current 2-generation study performed with a 70 day pre-pairing period in the P generation and a 123 day pre-pairing period in the F1 generation.

The HC data does not include individual animal data but the mean body weights of the parental F0 and F1 females in the prepairing, gestation and lactation period and the mean body weights of the F1 and F2 pups. In the Appendix 1 the range of historical mean body weight values and additionally the wider range of the individual data are listed. The individual range has been estimated as overall mean value ± the average standard deviation. Furthermore the correlating mean litter size is given for each study as well as the incidence of total litter losses.

HC data confirm inverse relationship of litter size and mean pup body weight and allows to derive correction factors

The existence of an inverse relationship of pup body weight development and litter size at least until culling at PND 4 due to competition for maternal milk is intensively described in Agnish & Keller, 1997 [Fundam. Appl. Toxicol. 38, 2–6]. This characteristic is also seen in the HC data: the pre-culling data at PND 1 and 4 shows the typical inverse relationship between litter size and mean pup weight development, represented by a linear trend line with negative slope as indicated in Figure 5.6/1 for the F1 generation and Figure 5.6/2 for the F2 generation below.

Figure 5.6/1 Historical control data: Correlation of mean litter size and mean pup weight at PND 1 and 4 in the F1-pup generation

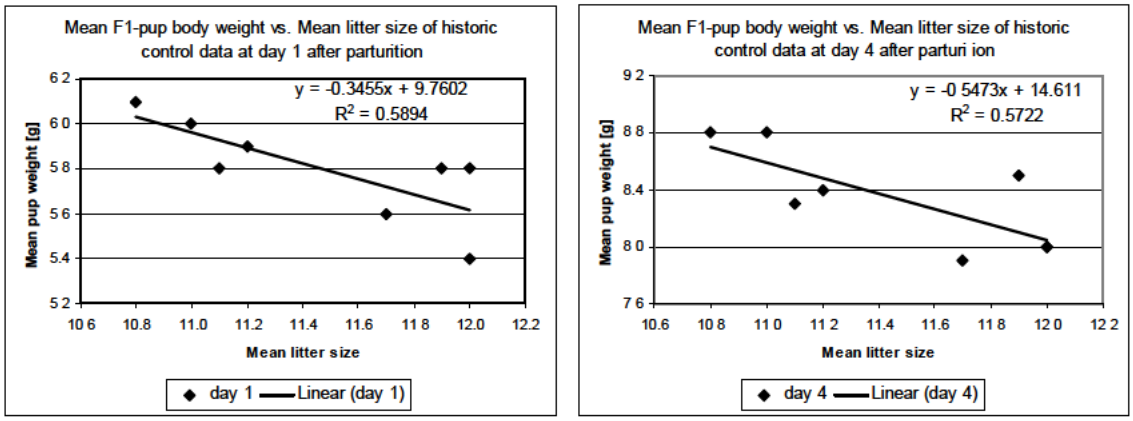
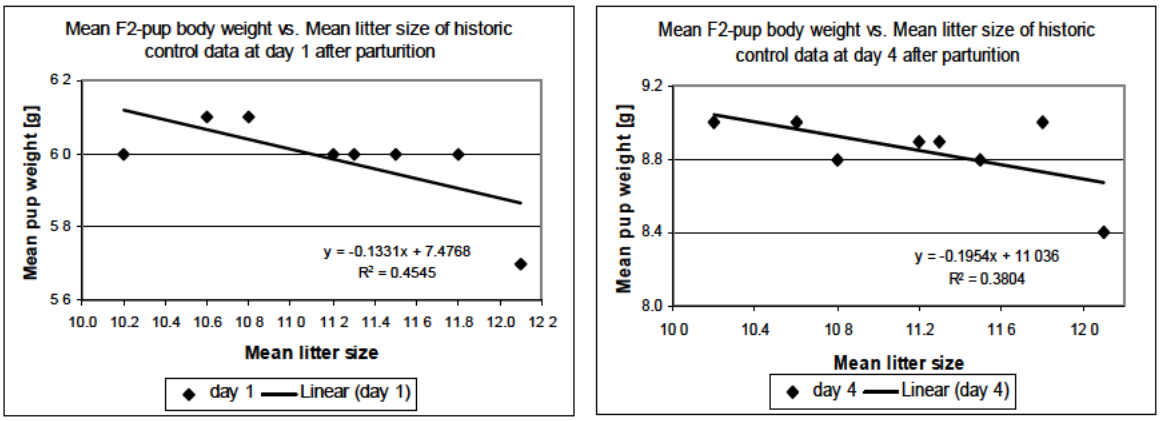


Figure 5.6/2 Historical control data: Correlation of mean litter size and mean pup weight at PND 1 and 4 in the F2-pup generation



Chahoud & Paumgartten,2009 [Environmental Research 109, 1021-1027] introduced an approach to standardize pup weight data for litter size effects by correction factors generated in a historical control cohort until the day of culling. This approach is described on the basis of individual data in the HC cohort to correct the individual data in the corresponding study. This approach was adopted to correct the mean values on the basis of the linear regression parameters generated in the Historical control data for day 1 and day 4 (Further information is given in the Appendix I).

The thereby derived correction factors were used to norm the mean pup weights found at day 1 and 4 for the F1-generation in the following discussion.

Discussion of the 2-generation study with BAS 351 H

F1 generation pup body weights in view of historical control data

All mean pup weights in the F1 generation of the mid and high dose group were significantly reduced compared to the concurrent control according to the statistical evaluation of the study report (marked bold in Table 5.6/5). The mean pup weights were most affected in the mid dose at day 4 and 7 with a -11.9 % reduction compared to control at day 4 and -10.2% at day 7; both values in addition were outside the historical control mean values. The high dose group showed the peak at day 7 with 8.8 % reduction vs. control. Furthermore, the pup weights of the high dose group were outside the historical control at day 4 with a -8.3 % reduction to the concurrent control. The later timepoints revealed the tendency of recovery and were additionally within the range of historical control means.

Table 5.6/5 Mean pup body weight in the F1 generation, sexes combined [g]: Comparison of findings in the BAS 351 H 2-generation reproduction study with historical control data (n=8)

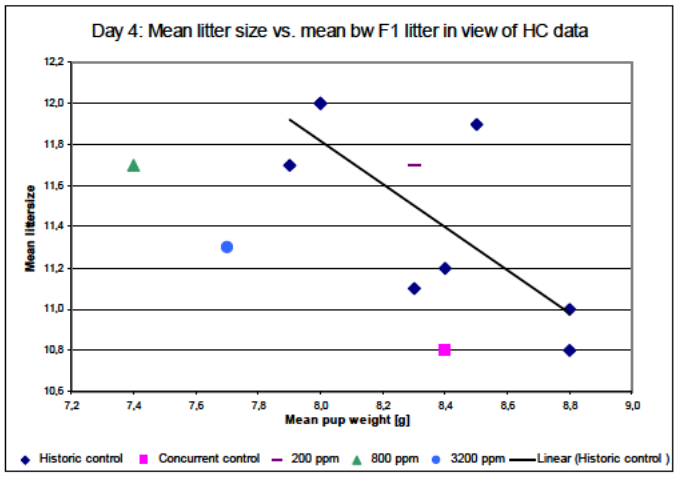
	F1 pups body weight during lactation [g]				Historical control F1-generation (n=191 litters)		
	Mean absolute values [g][1] (%Deviation to current control)				Range of body weight means [g] (n=8)		Individual range [g] Mean ± SD
Day	0 ppm	200 ppm	800 ppm	3200 ppm	Lowest	Highest	
1	5.9	5.7 (-3.4)	**5.4 (-8.5)**	**5.5 (-6.8)**	5.4	6.1	5.8 ± 0.7
4	8.4	8.3 (-1.2)	***7.4 (-11.9)***	***7.7 (-8.3)***	7.9	8.8	8.3 ± 1.1
7	13.7	13.4 (-2.2)	***12.3 (-10.2)***	**12.5 (-8.8)**	12.5	14.2	13.3 ± 1.8
14	28.6	28.8 (0.7)	**27.4 (-4.2)**	**26.9 (-5.9)**	24.4	29.6	27.6 ± 3.4
21	45.3	46.2 (2.0)	**43.1 (-4.9)**	**43.5 (-4.0)**	36.9	47.4	43.3 ± 5.4
Mean Litter size	10.8	11.7	11.7	11.3	11.5		

1) Statistically significantly reduced means as mentioned in the study report are given in **bold**.
2) Historical control values as calculated from Historical control data report 2011/1145234
Italic marked values are outside the historical control values.

A steady dose relationship is not evident in the F1 generation; neither in correlation to the 4-fold substance increase between the mid and high dose, nor in correlation to the substance increase during the lactation phase, which questions the relevance of the effect.

The illustration of the BAS 351 H pup weight mean values at PND 4 in relation to the historical control data, shown in Figure 5.6/3, reveals that all dose groups including the concurrent control show a lower weight at a respective litter size than expected in view of the historical control data. As the current study is not represented by the historical control data it is reasonable to use the concurrent control as basis for the calculation of deviation.

Figure 5.6/3 2-generation study with BAS 351 H in comparison to historical control data: Mean litter size vs. mean pup body weight in the F1 generation at day 4 after parturition



Following the approach of normalizing the pup weight means to eliminate litter size dependency by using the correction factors generated with the historical control data as explained in Appendix 1, the following normalized mean pup weights were generated for the time until culling, see Table 5.6/6.

Table 5.6/6 Normalized mean pup body weights in the F1 generation

Day	Mean pup body weight values [g] (% Deviation to respective control)			
	F1 pups body weight during lactation [g]			
	0 ppm	200 ppm	800 ppm	3200 ppm
1	5.7	5.8 (2%)	5.5 (-3%)	5.4 (-4%)
4	8.0	8.4 (5%)	7.5 (-7%)	7.6 (-5%)

In respect of the fact, that the deviation to control under litter size normalized conditions is reduced at the most relevant time point PND 4 from -11.9% to -7% for the mid dose and from -8.3 to -5% for the high dose, it is reasonable to consider that this is the maximal induced substance effect in the F1 generation, which is thereby clearly below the originally discussed values.

Further evidence that the pup weights are less substance affected than indicated in the original report is the comparison of the mean litter weights. The mean litter weights show at PND 1 no deviation to control and at PND 4 a reduction of 3 to 4% in the mid and high dose group.

Table 5.6/7 F1 mean litter weight at PND 1 and 4 [g]

Day	Mean values ± SD [g] / (%Deviation to current control) [1]			
	0 ppm	200 ppm	800 ppm	3200 ppm
1	63.7 ± 12.4	66.5 ± 10.2 (+4.4)	63.8 ± 13.4 (+ 0.1)	63.0 ± 11.2 (-1.1)
4	88.6 ± 14.9	96.1± 13.2 (+8.5)	85.7 ± 20.3 (-3.2)	85.1 ± 18.0 (-3.9)

1) Mean values and Deviation from the control [%] calculated from reported absolute values.

Therefore, in the next step the individual data of the F1 pups and the corresponding dams were evaluated to look for maternal toxicity.

Discussion of pup body weight means in the F1 generation on the basis of individual F0-dams

In the Appendix 2 the cumulated food consumption data of the control dams during gestation (days 1 to 21) and during the lactation period (day 1 to 21) as well as the corresponding body weights at day 1 of the lactation phase were listed together with the mean pup weights on PND 1 to 21. This was done for all dose groups and the mean and individual food consumption data were compared with the concurrent control.

The historical control pup weight means ± the mean of the standard deviation were used as conservative approximation of the range of individual pup weights. All pup weight values below this individual range were checked for potential maternal effects in regard to food consumption and/or body weight effects. The range of individual pup weights in the historical control were calculated to be between 5.1, 7.2, 11.5, 24.2, 37.9 and 6.5, 9.4, 15.1, 31.0, 48.7, each at postnatal days 1, 4, 7, 14 and 21, respectively.

Discussion of the F0 dams breeding the F1 generation: concurrent control (Table 5.6/19)

Of the 25 control dams, three had no progeny. From the remaining 22 litters, 3 litters revealed isolated pup weight means below and 8 litters showed isolated pup weight values above the range of historical individual weights throughout the suckling period.

The mean maternal food consumption between PND 1 and PND 4 was 27.2 ± 4.9 g, and the corresponding mean body weight gain was 16.7 g in the first 4 days after parturition.

The litters with above historical control pup weight values were those with under average litter size (dam #101 with 8 pups, #104 with 6 pups, #107 with 9 pups, # 119 with 9 pups) or those of mid size and increased maternal food intake (# 105 and # 123 with 11 and 12 pups).

The litters of dams #109, #118 and #122 showed mean pup weights below the historical control range. #122 was the dam with the extraordinary high litter size of 18 pups. For dams #109 and #118 the reduced pup weight values were correlated either with reduced maternal food intake and/or body weight loss between PND 1 and 4 (#109 lost 4 grams and # 118 gained only 4 grams body weight between PND 1-4 instead of the mean body weight gain of 16.7 g). The highest maternal food reduction in comparison to the mean was – 26% in dam # 118 and -34% in dam # 112.

According to this control group a reduction in maternal food consumption after parturition of around -30 % is considered to be within the normal range of nutritional variability after parturition in individual cases.

Generally, the nutritional need is influenced by the litter size: Dams of small litters (7 or less pups) generally showed a slightly reduced food intake without showing reduced pup weights while dams of above-average litters (12 and more) showed rather slightly increased food intake. High numbers of pups (here 18 pups per litter) resulted in reduced mean pup weights even with increased food consumption.

Discussion of the F0 dams breeding the F1 generation: 200 ppm group (Table 5.6/20)

From the 25 litters, three revealed isolated pup weight means below the range of individual historical control weights (#130, #136, #149) and 9 litters showed isolated pup weight values above the historical control weight range. The higher pup weight means were in the most cases correlated with an increased maternal food intake. No animal with increased food intake, respectively increased substance intake, showed reduced pup weight means.

The mean maternal food consumption in this dose group between PND 1 and PND 4 was 28 ± 6.4 g, thereby slightly higher than that of the concurrent control. The mean body weight gain was 18.6 g, which is slightly above the control.

The litters with individual pup weight means below the historical individual range (#130, #136, #149) showed either a higher number of pups or a reduced average food consumption in the lactation phase. Nevertheless, the maternal food intakes ranged within the naturally occurring variability of the parameter found in the control group.

Discussion of the F0 dams breeding the F1 generation: 800 ppm group (Table 5.6/21)

In the 800 ppm group the litter size pattern was clearly shifted towards higher litter sizes, with only one litter with 8 or less pups, 12 litters with 9 -12 pups and 9 litters with 13 and more pups. This shift towards higher litter size is one reason for a reduced mean pup weight gain especially at PND 1 to 7.

The mean maternal food consumption between PND 1 and PND 4 is stated in the report to be 25.5 g (- 5.9 % of control) under exclusion of 2 dams (# 159 and #164) that had complete litter losses at PND 4. These dams showed the most significant food reduction between PND1 and PND4 with only 3 g and 10 g food intake per day instead of normally 27.2 g, which is a food intake of only 11% or 37% of the concurrent control. Both dams showed marked toxicity in this sensitive period indicated by a body weight loss of 5 g or 26 g between PND1 and PND4. These dams were excluded in the report from the evaluation as they did not rear their progeny over the whole lactation phase. If these pups are included into the calculation for the food intake up to PND 4, the food consumption in the respective interval is decreased to 23.8 g, thereby about 12.5 % lower than the concurrent control. Nevertheless, this reduction shows no significance. This is because there is a high deviation in this time interval which is contributed to some animals with more than 50 % increase in nutritional intake between day 1 and 4 (#160, 162, 170- none of those showed a reduced pup weight after PND 1 which would be indicative for a direct substance induced effect on the pups); and those with a strong decrease in nutritional intake – all showed a more or less pronounced reduction of pup weight.

Taking only those dams into account which had a total litter loss or whose pups showed reduced pup weights over the period of PND 4-7 which are #152, 155, 156, 159, 164, 167, 171, 172 and 175, the mean food consumption between PND 1 and 4 is 14.2 ± 9.6g (see Table 5.6/8), thereby significantly reduced about 48% under the concurrent control and the body weight gain would be reduced to 4.8 g between PND 1 and 4, which is only 29 % of the concurrent control. A further focusing on only those dams that showed a body weight reduction based on maternal toxicity and not those that had reduced pup weight based on high litter size would additionally eliminate dam # 152 and #172. This furthermore reduces the food consumption to a value of 10.4 ± 6.7g (38.4% of control) and the mean maternal body weight gain to -1.7 g between PND1 and 4.

This clearly demonstrates a pronounced maternal toxicity by significantly reduced food intake and weight gain values and the developmental toxicity of the pups is contributed to this maternal toxicity.

Table 5.6/8 P-generation nursing the F1 pups: 800 ppm group; Individual maternal food consumption data and body weight respective Body weight gain of the F0 dams and the corresponding F1 litter body weight means and Litter size

800 ppm Dam #	Food consumption [g/animal/day]								BW [g]	BWG [g]	Mean Pup weight [g]		
	Gestation		Lactation						maternal				
	Days 1-21	Dev.to Control	Days 1-4	Dev.to Control	Days 4-7	Dev.to Control	Days 7-14	Dev.to Control	Days 1	Days 1-4	Litter size	PND 1	PN D4
152	60	99%	25	92%	41	105%	47	92%	218	33	14	5.1	6.6
155	60	99%	5	18%	33	84%	49	96%	244	-9	13	5.4	4.8
156	67	111%	19	70%	35	89%	47	92%	253	7	15	4.9	6.3
159	70	116%	3	11%	-	-	-	-	258	-5	14	5.7	-
164	67	111%	10	37%	-	-	-	-	279	-26	5	5.5	-
167	62	103%	14	51%	32	82%	37	73%	263	4	9	5.8	5.5
171	65	108%	4	15%	28	71%	52	102%	261	-14	13	5.2	5.6
172	-	-	30	110%	42	107%	55	108%	259	22	15	4.9	6.8
175	55	91%	18	66%	62	158%	47	92%	224	31	11	5.0	6.0
Mean	63.3	105%	**14.2**	**52.3%**	39.0	99%	47.7	94%	**251.0**	**4.8**		**5.3**	**5.9**
								Dev. to control	**102%**	**29%**		**88%**	**69%**
Under exclusion of #152 and # 172 which showed reduced pup weight despite normal food intake													
Mean	63.7	105%	**10.4**	**38.4%**	38.0	97%	46.4	91%	**254.6**	**-1.7**		**5.3**	**5.6**
								Dev. to control	**103%**	**-10%**		**90%**	**66%**

Futhermore, the low pup weights are accompanied by malnutrition of the dams and therefore with a reduced substance intake. Otherwise the recovery of the mean pup weights towards the end of the lactation phase is accompanied with the increase in maternal food and substance intake, which indicates that the pup weight effects in this group are not substance-related but secondary to the maternal food consumption which is in the affected dams of this dose group far below the normally occurring variability.

Discussion of the F0 dams breeding the F1 generation: 3200 ppm group (Table 5.6/22)

The litter size pattern in this dose group is 2 litters with 8 or less pups, 14 litters with 9 -12 pups and 7 litters with 13 and more pups. Thus, also here a slight shift towards higher litter size is seen in this dose group in comparison to the control group.

The mean maternal food consumption between PND 1 and PND 4 was reported to be 26.8 g (-1.1 % deviation to control) also in this dose group under exclusion of 2 dams (# 191 and # 196) that showed marked feed refusal to below 4 g/animal /day (<15% of the concurrent control) which both showed distinctive low body weights of 181 and 185 g at PND 1 (ca. 25% below the mean concurrent control) and had total litter losses at PND 0 (partly cannibalized) or 4.

In this dose group a slightly different kind of maternal toxicity is seen, which is more affected by the general reduced maternal body weight during gestation and lactation. On day 1 after parturition the mean maternal body weight is significantly reduced about 9% compared to that of the concurrent control. Three dams, # 177 with +47%, #181 with + 25% and # 187 with + 36% had a higher feed intake than control and thereby a higher substance burden, but none of these dams had litters with reduced body weight at any time during the lactation period. Also here a higher substance burden did not directly affect the fitness of the pups.

Otherwise, a decrease in mean pup weight was seen in a chronological dependency of maternal nutritional intake in dams # 179, # 185, # 189 and # 198. These litters are considered to show reduced mean pup weight at some time interval as consequence of the reduced maternal food intake. For dam #183, whose litter showed reduced mean pup weight at PND 4, a correlation to the maternal food intake is impossible the documentation is missing. All other affected litters where those of higher pup count per litter, with 13 (# 188), 14 (# 194) and 15 pups (# 182) per litter and were affected in the typical time period of PND 1 to 7. Nice to see are the dependencies in these litters, for example the birth weight decreases for each additional pup and the impact of litter size on the magnitude and duration of pup weight decrease.

Thus, taking all points together for the F1-generation, the reduced pup weight at PND 4 and 7 at 800 and 3200 ppm is unlikely to be directly caused by BAS 351 H but a consequence of maternal toxicity. This is supported by the following findings:

- Overall the pup weight effects are correlated to either higher litter size or to significant reduced maternal feed intake within the early lactation phase.
- Although the group mean value of maternal feed intake of the mid dose does not significantly vary from the control group, the individual analysis of the data revealed relevant signs of toxicity.
- The at most affected litters were from dams that showed a significantly reduced food intake (-61.6%) between lactation day 1-4, thereby directly influencing their own body weight gain (mean of -1.7g within PND1-4 whereas the control showed a weight increase of 16.7g within this period) and the pup weights.
- The maternal body weight loss in single F0-dams has to be considered as severe maternal toxicity in this sensitive period of nursing.

- Those dams that had a high food intake and consequently a high substance burden showed no impaired pup development, which argues against direct toxicity to the pups.
- The mean pup weight at PND 14 and 21 was within the range of historic variability of this parameter. This allows the conclusion that in the period of the highest maternal substance burden the pups were recovering. The effect is therefore not considered to be mediated via the maternal milk which is additionally supported by the data of the milk transfer study in goat that showed no major substance transfer into this matrix.

F2 generation pup body weights in view of historical control data

The F2 generation is less affected than the F1 generation, showing statistically reduced mean pup body weights in the mid dose at PND 4, 14 and 21 and in the high dose from PND 4 to PND 21 (see Table 5.6/9). No significant reduction is seen at PND 1, which is an indication that Bentazone does not affect the pup body weight of the F2 generation during gestation. All pup body means of animals treated with BAS 351 H are within the range of the historical control values. Whereas the low and mid dose groups are from PND7 onwards rather above the average historic value, the 3200 ppm group is slightly and throughout the whole dosing period below the mean historical control value. The concurrent control is rather high, for day 4 and 21 even above the top border of the historical control means.

Table 5.6/9 Mean pup body weight means in the F2 generation, sexes combined [g]: Comparison of findings in the BAS 351 H 2-generation reproduction study with historical control data (n=8)

	F2 pups body weight during lactation [g]				Historical control F2-generation[2] (n=188 litters)		
	Mean absolute values [g] [1]/ (%Deviation to current control)				Range of body weight means [g] (n=8)		Individual range [g] Mean ± SD
Day	0 ppm	200 ppm	800 ppm	3200 ppm	Lowest	Highest	
1	6.0	6.0 (0)	5.9 (-1.7)	6.1 (+1.7)	5.7	6.1	6.0 ± 0.7
4	*9.1*	8.7 (-4.4)	**8.8 (-3.3)**	**8.6 (-5.5)**	8.4	9.0	8.9 ± 1.3
7	14.6	14.3 (-2.1)	14.3 (-2.1)	**14.0 (-4.1)**	13.2	14.7	14.2 ± 1.9
14	31.0	30.5 (-1.6)	**30.1 (-2.9)**	**28.5 (-8.1)**	26.0	31.3	29.5 ± 3.4
21	*50.7*	50.6 (-0.2)	**48.7 (-3.9)**	**46.5 (-8.3)**	42.3	50.5	47.9 ± 5.2
Mean Litter size	10.5	11.5	11.0	10.8	11.2		

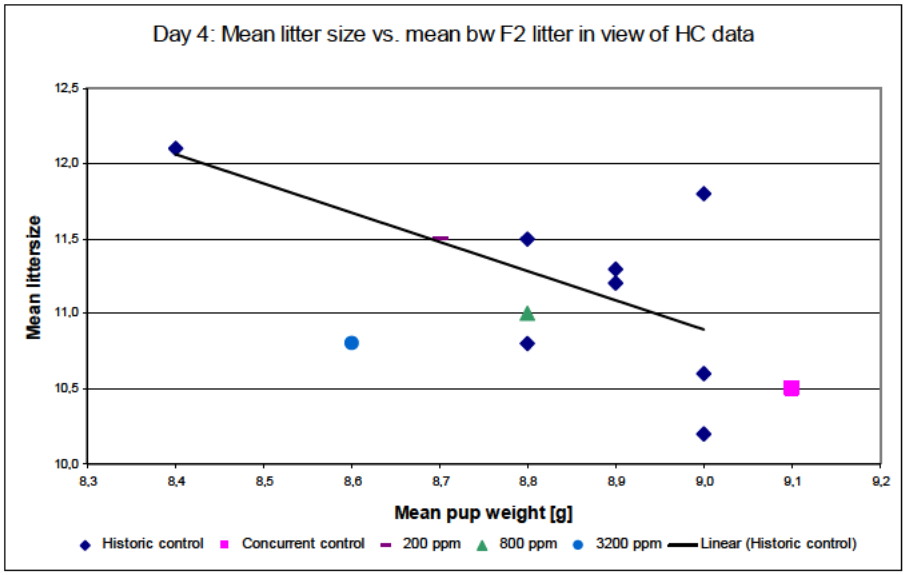
1) Statistically significantly reduced means as mentioned in the study report are given in **bold**.

2) Historical control values as calculated from Historical control data report 2011/1145234

Italic marked values are outside the range of historical control means.

The illustration of the BAS 351 H pup weight mean values at PND 4 in relation to the historical control data, shown in Figure 5.6/4, reveals that at day 4 the concurrent control is well in line with the historical control values, but in view of the mean litter size/pup weight relation displays an extreme under the controls. Therefore it is possible and reasonable to use the historical control mean as more representative average value than the concurrent control.

Figure 5.6/4 2-generation study with BAS 351 H in comparison to historical control data: Mean litter size vs. mean pup body weight in the F2 generation at day 4 after parturition



The calculation of the % deviation of pup weight means to the historical control (see Table 5.6/10) reduces the original values found in relation to the concurrent control to lower values, with a maximum in pup body weight reduction of -3.4% for the high dose. There is no clear dose-effect relationship. As far as in the high dose level maternal toxicity is seen over the complete gestation/lactation period, a potential pup body weight effect is reasonable to be attributed to this maternal toxicity. The mid dose group is considered to be free of any effect on pup body weight.

Table 5.6/10 Deviation of F2 pup body weight means during lactation in the BAS 351 H 2-generation reproduction study compared to the concurrent control and the historical control mean values

Day	Deviation from concurrent control [%] [1]			Deviation from HC Mean [%] [2]		
	200 ppm	800 ppm	3200 ppm	200 ppm	800 ppm	3200 ppm
1	0.0	-1.7	1.7	0.2	-1.5	1.9
4	-4.4	-3.3	-5.5	-1.7	-0.6	-2.8
7	-2.1	-2.1	-4.1	0.7	0.7	-1.4
14	-1.6	-2.9	-8.1	3.3	2.0	-3.4
21	-0.2	-3.9	-8.3	5.7	1.7	-2.9

1) Deviation from the control [%] calculated from reported absolute values.

2) Deviation to Historical controls (HC) mean values [%] calculated from reported absolute values and the mean historical controls, see Appendix 1

Thus; taking all points together for the F2-generation, the apparent deviation in the pup weights between the treated and control groups is not substance induced, but result from abnormally high control pup weights. If the historical control was considered as the relevant reference the deviation of the mid dose pup weight is eliminated and the only remaining effect is a slight pup weight reduction from PND 4 onwards with a maximum at PND 14 of -3.4 % in the high dose group.

Conclusion

The observed effects on pup weight in the **F1 generation** are to an extent to the smaller litter size of the control group. A correction for the litter size reduced the pup weight deviations to the concurrent control at the most affected time point PND 4 to a maximum of -7% in the mid dose and -5% in the high dose.

The remaining deviation in pup weight development has been shown to be the result of maternal toxicity induced by BAS 351 H in individual dams already manifested at the mid dose. Although the group mean values of the mid dose do not significantly vary from the control group, the individual analysis of the data showed that the litters with significantly reduced pup weights arise from dams showing relevant signs of toxicity as demonstrated by a transient food refusal within the early lactation phase and a severe maternal body weight loss or body weight gain reduction. Focussing only on those dams with clearly affected litters revealed reduced mean maternal food consumption between PND 1 and 4 to 38 % of the concurrent control and a mean body weight loss of 1.7 g within PND1 and 4 in comparison to the control group that gained in this sensitive time frame about 16.7 g. The respective pup body weight development is most likely impaired as a consequence of this nutritional deficit. A recovery was observed in dams and pups towards the end of the lactation phase, where the food consumption levels were comparable to those of the control values. In the high dose group a maternal body weight reduction was apparent in all periods of the study and the reduction of pup weight was shown to be likewise attributed to a high litter size and/or a reduced maternal food intake.

For the **F2 generation** it was demonstrated that the mean litter size of the concurrent control group (10.5 pups/litter) was well below the historical control average value (11.2 pups/litter) and close to the lower range of the historical control data (10.2 pups/litter). The low mean litter size of the control group led to higher mean pup weights in this group exceeding the upper range of the historical control data. The mean pup weights of the treated groups, attributed to mean litter sizes of 11.0 and 10.8 pups/litter for the mid and high dose, respectively, were at any time within the historical control data range. Thus; the apparent deviation in the pup weights between the treated and control groups is not likely to be substance induced, but result from abnormally high control pup values. If the historical control was considered as the relevant reference the deviation of the mid dose pup weight is eliminated and the only remaining effect is a slight pup weight reduction from PND 4 onwards with a maximum at PND 14 of -3.4 % in the high dose group.

Additional arguments that Bentazone is not directly acting on the pups are the facts that initial effects were seen at PND 4 and not directly after parturition, and that the F2 generation is less susceptible than F1. Furthermore, pups from dams having a high food consumption did not show any adverse effects despite the higher substance intake.

Overall, the F1 and F2 generations are not more susceptible than the respective parental generation. Alterations are observed only at parentally toxic levels.

Therefore the following relevant effect levels in ppm and mg/kg bw/day (according to the substance intake data shown in Appendix 3) are considered appropriate in the 2-generation study with BAS 351 H:

- **NOAEL** (maternal and developmental): 200 ppm (equivalent to 22 mg/kg bw/day based on the mean substance intake of F0-dams between PND1-4 during lactation)

- **LOAEL** (maternal and developmental): 800 ppm (equivalent to 80 mg/kg bw based on the mean substance intake of F0-dams between PND1-4 during lactation)

- The resulting effect levels were derived on the basis of:

    - **Maternal toxicity** is evident for the F0 females especially between PND 1 and 4 in the mid and high dose group (800 and 3200 ppm) and in the F1 females only in the high dose group (3200 ppm)

    - **Reproductive/developmental toxicity** is based on the slightly decreased pup body weights seen at PND 4 and 7 for the F1 pups in the mid and high dose (800 and 3200 ppm) and for the F2 pups in the high dose group **secondary** to maternal toxicity

APPENDIX 1:

Historical control data derived from report 2011/1145234

Historical control data consists of 8 studies performed at [redacted] within the period of 1984 and 1988 under a similar protocol, all preformed as diet studies in Wistar/Han rats, outbred, [redacted]. Grey highlighted is the 2-generation study with Bentazone, project nr 67757, demonstrating, that the Historical control database does represent the relevant time period. Data are taken from the Historical control report Doc ID 2011/1145234 [redacted] 2011).

Table 5.6/11 Study identification and study period in Historical control data

HCD No.	Report No.	Project	Study	Start	End
Study 1	13	30644	2-Gen	17.2.1984	28.12.1984
Study 2	3	36911	2-Gen	1.3.1985	29.12.1985
Study 3	14	48846	2-Gen	3.1.1986	16.11.1986
Study 4	16	61975	2-Gen	17.10.1986	31.8.1987
	17	67757	2-Gen	7.11.1986	1.10.1987
Study 5	22	71561	2-Gen	12.12.1986	28.10.1987
Study 6	20	71504	2-Gen	9.1.1987	24.11.1987
Study 7	32	71583	2-Gen	20.3.1987	1.3.1988
Study 8	31	201205	2-Gen	15.7.1988	22.6.1989

In the following table relevant study parameters regarding the duration of pre-pairing treatment, gestation and lactation period for the P and F1 generation demonstrate similar conditions, same route of application and same species.

Table 5.6/12 Treatment interval in the 8 studies used as Historical control data

HCD No.	P Generation			F1-Generation		
	Pre-pairing	Gestation	Lactation	Pre-pairing	Gestation	Lactation
Study 1	1 - 68	0 - 21	0 - 21	1 - 123	0 - 21	0 - 21
Study 2	1 - 56	0 - 21	0 - 21	1 - 119	0 - 21	0 - 21
Study 3	1 - 56	0 - 21	0 - 21	1 - 124	0 - 21	0 - 21
Study 4	1 - 70	0 - 21	0 - 21	1 - 122	0 - 21	0 - 21
	1 - 70	0 - 21	0 - 21	1 - 123	0 - 21	0 - 21
Study 5	1 - 70	0 - 21	0 - 21	1 - 125	0 - 21	0 - 21
Study 6	1 - 70	0 - 21	0 - 21	1 - 126	0 - 21	0 - 21
Study 7	1 - 70	0 - 21	0 - 21	1 - 126	0 - 21	0 - 21
Study 8	1 - 70	0 - 21	0 - 21	1 - 101	0 - 21	0 - 21

Range of mean and individual body weights

In the following tables the range of mean body weights of the F0/F1-females during gestation and lactation and that of the F1/F2-pups as well as the range of individual body weights calculated as mean of means ± mean of the respective standard deviations is presented.

Table 5.6/13 Historical control data: Range of mean and individual body weight [g] of F0/F1-females during gestation[1]

	F0-generation (n=196 individuals)			F1-generation (n=191 individuals)		
Day	Range of body weight means (n=8) [g]		Individual range [g]	Range of body weight means (n=8) [g]		Individual range [g]
	Lowest	Highest		Lowest	Highest	
0	226	242	232 ± 19	248	275	261 ± 21
7	244	261	251 ± 19	263	297	279 ± 22
14	266	284	275 ± 20	289	317	302 ± 23
21	324	347	335 ± 25	351	384	366 ± 29

1) The values are calculated based on the values reported in the HC report 2011/1145234

Table 5.6/14 Historical control data: Range of mean and individual body weight [g] of F0/F1-females during lactation1

	F0-generation (n=193 individuals)			F1-generation (n=188 individuals)		
Day	Range of body weight means (n=8) [g]		Individual range [g]	Range of body weight means (n=8) [g]		Individual range [g]
	Lowest	Highest		Lowest	Highest	
1	**232**	**256**	**244 ± 20**	**261**	**292**	**273 ± 25**
4	**251**	**276**	**263 ± 21**	**281**	**306**	**290 ± 23**
7	**260**	**289**	**273 ± 21**	**286**	**314**	**297 ± 23**
14	**278**	**299**	**286 ± 21**	**301**	**322**	**309 ± 22**
21	**268**	**292**	**279 ± 20**	**298**	**286**	**313 ± 21**

1) The values are calculated based on the values reported in the HC report 2011/1145234

Table 5.6/15 Historical control data: Range of mean and individual F1/F2-pup body weights [g] (males & females)1

Day	F1-generation (n=193 litters)			F2-generation (n=188 litters)		
	Range of body weight means [g] (n=8)		Individual range [g] Mean ± SD	Range of body weight means [g] (n=8)		Individual range [g] Mean ± SD
	Lowest	Highest		Lowest	Highest	
1	5.4	6.1	5.8 ± 0.7	5.7	6.1	6.0 ± 0.7
4	7.9	8.8	8.3 ± 1.1	8.4	9.0	8.9 ± 1.3
7	12.5	14.2	13.3 ± 1.8	13.2	14.7	14.2 ± 1.9
14	24.4	29.6	27.6 ± 3.4	26.0	31.3	29.5 ± 3.4
21	36.9	47.4	43.3 ± 5.4	42.3	50.5	47.9 ± 5.2

1) The values are calculated based on the values reported in the HC report 2011/1145234

Generation of correction factors to standardize pup weights for the litter size
The mean body weights of the F1-pups at pre-culling day 1 and 4 in respect of the corresponding mean litter size were used for linear regression. The equation is given in the table below in the last column.

Table 5.6/16 Historic control data: Correlation between litter size and pup body weight development [g] in the F1-pup generation (males & females)

		Study 1	Study 2	Study 3	Study 4	Study 5	Study 6	Study 7	Study 8	Mean	Linear Regression
Mean Litter size at day 1		11.1	11.9	12.0	11.7	11.2	12.0	11.0	10.8	11.5	Mean Pup weight = function (Littersize)
Mean pup body weight [g]	day 1	5.8	5.8	5.4	5.6	5.9	5.8	6.0	6.1	5.8	y =-0.3455 x + 9.7602 R = 0.5894
	day 4	8.3	8.5	8.0	7.9	8.4	8.0	8.8	8.8	8.3	y =-0.5473 x + 14.611 R= 05722
	day 7	13.3	12.5	13.1	12.9	13.4	12.8	14.0	14.2	13.3	Post culling period
	day 14	28.6	24.4	27.3	27.3	27.1	27.5	29.6	29.2	27.6	
	day 21	45.6	36.9	41.3	41.0	43.1	45.7	47.4	45.4	43.3	

According [redacted] (2009, see DocID 2011/1262290) “The correction factor Cn should be determined from the historical control data for a particular rat strain using the expression: Cn=Ym/Yn, where Ym is the average pup body weight for the most frequent litter size, and Yn the average pup body weight for a given litter with n pups. The pup body weight values should then be transformed by using the correction factors before making statistical comparisons.”

The litter size in the HC data F1 generation ranges from 10.8 to 12.0, with a mean value of 11.5 which is therefore used as standard litter size in the F1 generation. Litters of that size are not corrected for the corresponding pup weight, whereas litters with higher or lower litter size are corrected for the pup weight. The linear regression allows approximating pup weight data at the mean litter sizes found in the BAS 351 H study, which are 10.8, 11.7 and 11.3 for the F1 generation. The derived correction factors to be used for the F1 generation data at PND 1 and 4 are presented in Table 5.6/17.

Table 5.6/17 Correction factors (Cn) for the relevant litter sizes found in the 2-generation study with BAS 351 H for postnatal day 1 and 4, based on linear approximation of the HC data

Mean litter size x	Mean pup weight as function of littersize			
	Day 1, F1		Day 4, F1	
	y = - 0.3455x + 9.7602		y = - 0.5473x + 14.611	
	Calculated Pup weight Yn	Correction factor Cn=Ym/Yn	Calculated Pup weight Yn	Correction factor Cn=Ym/Yn
10.8	6.0	0.9599	8.7	0.9560
11.3	5.9	0.9882	8.4	0.9870
11.5	5.8 (=Ym)	1	8.3 (=Ym)	1
11.7	5.7	1.0121	8.2	1.0133

These correction factors were used to normalize the mean pup weights found at day 1 and 4 as given in the following table.

Table 5.6/18 Derivation of normalized mean pup weights

Day	Mean pup body weight values [g] (% Deviation to respective control)			
	F1 pups body weight during lactation [g]			
	0 ppm	200 ppm	800 ppm	3200 ppm
Litter size	10.8	11.7	11.7	11.3
Original values				
1	5.9	5.7 (-3.4)	5.4 (-8.5)	5.5 (-6.8)
4	8.4	8.3 (-1.2)	7.4 (-11.9)	7.7 (-8.3)
Correction factors				
Cn day 1	0.9599	1.0121	1.0121	0.9882
Cn day 4	0.9560	1.0133	1.0133	0.9870
Normalized values				
1	5.7	5.8 (2%)	5.5 (-3%)	5.4 (-4%)
4	8.0	8.4 (5%)	7.5 (-7%)	7.6 (-5%)

APPENDIX 2:

F0-Generation breeding for F1 litters: Individual maternal food consumption data during gestation and lactation and referring body weight development in F0 parental females in the lactation phase compared with the mean pup body weights of the corresponding litters at day 1, 4, 7, 14 and 21

Bold marked are pup body weights below the lowest value of the Historical control individual range, **Italic style** marks the pup body weights above the highest value of the Historical control individual range. The Historical control individual range was calculated as Mean over Means ± Mean of standard deviation
The values might differ from those in the report because they were calculated on the basis of the rounded values listed in the report. No value was excluded except those of non pregnant females.

Table 5.6/19 Control group (0 ppm); Individual food consumption data and Body weight respective BWG of the F0 dams and the corresponding F1 litter body weight means and Litter size

0 ppm Dam #	Food consumption [g/animal/day]								Maternal BW [g]		BWG [g]		Litter size	Mean Pup body weight [g]				
	Gestation		Lactation						maternal									
	Days 1-21		Days 1-4		Days 4-7		Days 7-14		Day 1		Days 1-4			Day 1	Day 4	Day 7	Day 14	Day 21
	g	% of mean	g	% of mean	g	% of mean	g	% of mean	g									
101	64	106%	25	92%	37	94%	48	94%	264		8		8	6.6	*9.8*	14.2	28.2	45.6
102	64	106%	28	103%	42	107%	51	100%	237		21		11	5.7	8.5	14.2	29.4	45.8
103	60	99%	40	147%	-	'-	52	102%	230		32		13	5.7	8.1	13.5	29.5	47.5
104	55	91%	22	81%	31	79%	42	83%	238		4		6	7.1	*10.6*	*15.4*	29.9	47.9
105	60	99%	26	96%	39	99%	56	110%	254		12		11	5.6	8.3	13.2	30.3	*49.2*
106	61	101%	25	92%	41	105%	53	104%	226		29		8	5.7	9.4	13.4	27.5	41.2
107	59	98%	32	118%	43	110%	56	110%	268		20		9	6.5	*9.8*	*15.8*	30.6	49.6
108	57	94%	30	110%	40	102%	52	102%	230		11		11	5.8	8.3	12.9	28.1	45.6
109	68	113%	-	-	-	-	-	-	252		-4	a)	11	5.4	**6.9**	**11.3**	**23.1**	**37.3**
110	-	-	-	-	-	-	-	-	-		-	b)	-	-	-	-	-	-
111	64	106%	32	118%	45	115%	57	112%	252		30		13	6.1	8.3	14.9	29.9	45.3
112	53	88%	18	66%	30	77%	47	92%	209		9		7	5.7	8.5	12.3	27.2	45.0
113	59	98%	-	-		0%		0%	-		-	c)	-	-	-	-	-	-
114	59	98%	25	92%	43	110%	45	88%	239		24		12	6.0	8.2	14.6	28.1	39.4
115	66	109%	32	118%	42	107%	54	106%	273		10		10	6.7	*9.8*	*15.3*	30.4	43.0
116	57	94%	27	99%	40	102%	49	96%	228		22		11	6.1	8.5	13.6	*32.6*	*52.8*
117	-	-	-	-	-	-	-	-	-		-	d)	-	-	-	-	-	-
118	55	91%	20	74%	30	77%	29	57%	248		4		12	5.4	**6.9**	**11.4**	**23.1**	41.1
119	61	101%	21	77%	29	74%	45	88%	272		8		9	6.7	*9.9*	15.0	31.0	44.0
120	54	89%	30	110%	45	115%	51	100%	232		28		10	6.3	9.0	14.9	30.3	47.8
121	63	104%	30	110%	44	112%	54	106%	230		31		13	5.2	7.5	13.0	27.5	47.3
122	68	113%	30	110%	44	112%	60	118%	256		24		18	5.3	**6.8**	12.2	29.6	47.3
123	65	108%	25	92%	40	102%	58	114%	273		16		12	6.4	8.6	13.2	28.8	*48.9*
124	57	94%	26	96%	40	102%	44	86%	241		20		12	6.1	8.5	13.4	25.8	39.7
125	-	-	27	99%	39	99%	66	130%	284		8	a)	11	5.3	8.0	13.8	30.0	48.1
Mean	60.4		27.2		39.2		50.9		247.1		16.7		10.8	6.0	8.6	13.7	28.7	45.4
±SD	± 4.4		± 4.9		± 5.2		± 7.7											

a) Food consumption not mentioned without explanation
b) Not pregnant
c) Did not give birth
d) Female was not mated

Table 5.6/20 200 ppm group; Individual food consumption data and Body weight respective BWG of the F0 dams and the corresponding F1 litter body weight means and Litter size

200 ppm Dam #	Food consumption [g/animal/day]								Maternal BW [g]		BWG [g]		Litter size	Mean Pup body weight [g]				
	Gestation		Lactation						Lactation									
	Days 1-21		Days 1-4		Days 4-7		Days 7-14		Day 1		Days 1-4							
	g	Dev.to Control	g	Dev.to Control	g	Dev.to Control	g	Dev.to Control	g	Dev.to Control				Day 1	Day 4	Day 7	Day 14	Day 21
126	52	86%	21	77%	32	82%	47	92%	209		16		8	5.3	8.6	12,4	25,8	44,9
127	60	99%	28	103%	39	99%	48	94%	217		33		12	6.0	8.3	13,4	25,7	42,8
128	54	89%	28	103%	37	94%	49	96%	237		17		9	6.1	*9.6*	14,0	27,8	42,0
129	69	114%	-	-	42	107%	60	118%	258		27	a)	13	6.0	8.4	13,8	*31,3*	*52,5*
130	-	-	22	81%	34	87%	47	92%	231		2	a)	10	6.0	8.0	11,5	**23,5**	40,5
131	63	104%	29	107%	44	112%	55	108%	256		18		12	5.2	8.1	13,9	32,3	*52,0*
132	60	99%	20	74%	38	97%	55	108%	260		5		12	5.3	8.0	13,3	30,0	*48,9*
133	59	98%	30	110%	40	102%	55	108%	264		10		8	6.0	*9.8*	*15,2*	*31,1*	*49,6*
134	63	104%	34	125%	40	102%	54	106%	243		24		12	5.8	8.6	13,6	28,8	45,9
135	57	94%	33	121%	42	107%	53	104%	236		34		13	5.6	8.3	14,3	29,2	47,1
136	58	96%	21	77%	40	102%	52	102%	244		28		13	5.4	7.2	**11,2**	28,1	47,5
137	71	118%	47	173%	43	110%	63	124%	279		13		15	6.1	8.8	14,3	*31,6*	*50,2*
138	-	-	26	96%	39	99%	50	98%	237		14	a)	10	6.2	8.2	12,9	26,3	43,3
139	63	104%	30	110%	36	92%	58	114%	251		28		12	6.4	*9.6*	14,0	29,6	*50,5*
140	59	98%	30	110%	42	107%	53	104%	236		21		10	6.0	8.7	13,2	27,5	43,8
141	53	88%	-	-	46	117%		0%	217		20	a)	11	5.4	8.1	12,7	27,7	44,4
142	56	93%	24	88%	36	92%	57	112%	264		6		11	6.1	*9.7*	*15,4*	*31,9*	*51,8*
143	60	99%	20	74%	36	92%	51	100%	246		5		11	5.4	8.0	13,5	29,2	47,8
144	65	108%	36	132%	31	79%	55	108%	261		5		12	5.6	7.5	12,0	27,8	43,1
145	63	104%	30	110%	47	120%	55	108%	242		29		12	5.7	8.0	14,1	29,6	43,5
146	62	103%	18	66%	40	102%	51	100%	251		5		13	5.8	7.8	13,3	29,3	42,7
147	60	99%	25	92%	39	99%	53	104%	272		7		13	5.2	7.4	13,0	28,4	44,8
148	62	103%	31	114%	43	110%	56	110%	236		31		12	6.0	8.7	15,1	*31,3*	*50,6*
149	62	103%	29	107%	40	102%	52	102%	235		27		15	**5.0**	**7.0**	12,0	27,9	45,5
150	60	99%	31	114%	45	115%	57	112%	251		40		13	5.2	7.5	13,6	29,1	41,3
Mean	60.5	100%	28.0	103%	39.6	101%	53.6	101%	245.3	99%	18.6		11.7	5.7	8.3	13.4	28,8	46,3
± SD	4.5		6.4		4.1		4.0						% control	96%	97%	98%	100%	102%

a) Food consumption not mentioned without explanation

Table 5.6/21 800 ppm group; Individual food consumption data and Body weight respective BWG of the F0 dams and the corresponding F1 litter body weight means and Litter size

800 ppm Dam #	Food consumption [g/animal/day]								Maternal BW [g]		BWG [g]		Litter size	Mean Pup body weight [g]				
	Gestation		Lactation						Lactation									
	Days 1-21		Days 1-4		Days 4-7		Days 7-14		Day 1		Days 1-4			Day 1	Day 4	Day 7	Day 14	Day 21
	g	Dev.to Control	g	Dev.to Control	g	Dev.to Control	g	Dev.to Control	g	Dev.to Control								
151	-	-	-	-	-	-	-	-	-	-	-	a)						
152	60	99%	25	92%	41	105%	47	92%	218		33		14	**5.1**	**6.6**	**11.2**	**23.9**	**36.8**
153	57	94%	24	88%	35	89%	47	92%	237		8		11	5.2	7.7	12.1	26.9	42.9
154	68	113%	24	88%	37	94%	47	92%	254		16		12	5.9	7.8	12.2	28.2	48.5
155	60	99%	5	18%	33	84%	49	96%	244		-9		13	5.4	**4.8**	**7.9**	23.9	40.7
156	67	111%	19	70%	35	89%	47	92%	253		7		15	**4.9**	**6.3**	**11.0**	24.6	38.0
157	58	96%	21	77%	36	92%	48	94%	221		12		11	5.3	7.8	12.5	25.8	42.8
158	59	98%	11	40%	30	77%	41	81%	233		-6		5	5.4	9.1	14.1	29.7	*49.5*
159	70	116%	3	11%	-	-	-	-	258		-5	b)	excluded from evaluation					
160	66	109%	41	151%	37	94%	61	120%	222		34		13	6.0	8.7	14.6	*32.5*	*52.2*
161	83	137%	32	118%	37	94%	56	110%	299		2		11	6.3	*9.8*	*15.6*	*34.1*	*54.6*
162	59	98%	48	177%	39	99%	50	98%	245		24		11	5.8	8.8	14.2	29.5	38.2
163	63	104%	26	96%	37	94%	50	98%	259		22		10	5.7	8.5	12.9	28.5	46.2
164	67	111%	10	37%	-	-	-	-	279		-26	b)	excluded from evaluation					
165	62	103%	32	118%	42	107%	54	106%	258		27		10	6.5	*9.7*	*15.5*	*31.4*	*51.4*
166	60	99%	27	99%	39	99%	52	102%	249		31		14	**5.1**	7.3	11.7	26.9	42.7
167	62	103%	14	51%	32	82%	37	73%	263		4		9	5.8	**5.5**	**9.1**	**23.3**	38.6
168	55	91%	21	77%	36	92%	52	102%	235		16		12	5.5	8.0	13.7	29.1	45.9
169	60	99%	35	129%	44	112%	57	112%	232		33		15	5.2	8.2	14.1	30.4	42.9
170	62	103%	41	151%	42	107%	61	120%	253		38		13	**4.9**	7.3	12.2	28.3	47.1
171	65	108%	4	15%	28	71%	52	102%	261		-14		13	5.2	5.6	9.2	24.4	43.1
172	-	-	30	110%	42	107%	55	108%	259		22	c)	15	**4.9**	**6.8**	**12.2**	26.8	38.5
173	63	104%	24	88%	35	89%	49	96%	233		10		12	5.7	7.3	12.2	25.6	40.8
174	62	103%	36	132%	32	82%	47	92%	240		35		12	5.6	7.5	12.3	25.7	**36.6**
175	55	91%	18	66%	62	158%	47	92%	224		31		11	**5.0**	**6.0**	**10.3**	23.8	**36.1**
Mean	62.7	104%	23.8	87.5%	37.8	96%	50.3	99%	247.0	100%	14.4		11.7	5.5	7.5	12.3	27.4	43.4
± SD	5.9		12.1		6.8		5.7						% control	92%	88%	90%	96%	95%

a) Not pregnant
b) All pups were missing on day 4 p.p.
c) Food consumption not mentioned without explanation

Table 5.6/22 3200 ppm group; Individual food consumption data and Body weight respective BWG of the F0 dams and the corresponding F1 litter body weight means and Litter size

3200 ppm Dam #	Food consumption [g/animal/day]								Maternal BW [g]		BWG [g]		Litter size	Mean Pup body weight [g]				
	Gestation		Lactation						Lactation									
	Days 1-21		Days 1-4		Days 4-7		Days 7-14		Day 1		Days 1-4			Day 1	Day 4	Day 7	Day 14	Day 21
	g	Dev.to Control	g	Dev.to Control	g	Dev.to Control	g	Dev.to Control	g	Dev.to Control								
176	57	94%	31	114%	39	99%	50	98%	214		28		12	5.7	8.1	12.7	28.0	45.9
177	74	122%	40	147%	40	102%	53	104%	273		9		14	6.3	9.0	14.6	*32.9*	*52.8*
178	59	98%	27	99%	37	94%	47	92%	227		18		8	6.2	9.2	13.3	29.2	*49.4*
179	49	81%	22	81%	33	84%	43	84%	206		18		10	5.4	7.3	12.1	**24.0**	38.6
180	54	89%	24	88%	36	92%	49	96%	218		13		9	6.0	8.4	12.7	25.3	41.1
181	59	98%	34	125%	36	92%	50	98%	213		20		10	5.8	8.5	13.0	28.1	44.4
182	65	108%	31	114%	40	102%	52	102%	248		35		15	**4.8**	**6.2**	**10.8**	26.4	43.6
183	55	91%	-	-	-	-	49	96%	240		16	a)	11	5.6	7.0	11.7	26.4	41.6
184	65	108%	24	88%	43	110%	51	100%	223		29		13	5.5	7.7	13.1	26.0	42.2
185	48	79%	20	74%	30	77%	40	79%	219		18		6	**5.0**	**6.6**	**9.6**	**21.6**	**37.1**
186	50	83%	23	85%	37	94%	43	84%	211		26		10	6.2	8.5	13.8	25.9	43.6
187	72	119%	37	136%	57	145%	49	96%	260		26		13	5.7	8.7	12.9	*32.8*	*53.0*
188	62	103%	30	110%	42	107%	49	96%	251		24		13	5.4	**7.0**	11.7	28.1	45.9
189	56	93%	14	51%	36	92%	47	92%	199		13		13	**4.6**	**4.9**	**8.4**	**21.1**	**35.7**
190	70	116%	28	103%	43	110%	56	110%	243		27		12	6.2	8.6	14.3	30.2	48.4
191	51	84%	1	4%	5	13%	19	37%	181		-8	b)	excluded from evaluation					
192	59	98%	21	77%	36	92%	45	88%	225		8		10	5.4	7.8	12.3	25.0	39.2
193	62	103%	26	96%	37	94%	49	96%	242		13		11	5.6	8.5	13.6	28.7	43.5
194	62	103%	20	74%	40	102%	52	102%	232		31		14	**5.0**	**5.8**	**10.0**	25.6	40.7
195	66	109%	30	110%	44	112%	58	114%	223		31		11	5.6	8.1	13.5	28.5	46.9
196	55	91%	4	15%	11	28%	19	37%	185		1	c)	excluded from evaluation					
197	58	96%	25	92%	35	89%	50	98%	231		25		13	5.4	8.0	13.4	28.0	44.4
198	53	88%	26	96%	38	97%	46	90%	212		21		11	**5.1**	7.7	12.0	**24.0**	**37.4**
199	60	99%	28	103%	41	105%	56	110%	234		27		12	5.4	7.7	12.4	28.4	44.8
200	66	109%	29	107%	39	99%	49	96%	218		23		10	*6.8*	9.3	14.0	27.0	43.8
Mean	59.5	98%	24.8	91%	36.5	93%	46.8	92%	225.1	91%	19.7		11.3	5.6	7.8	12.4	27.0	43.6
± SD	7.0		8.9		8.9		8.9						%control	94%	91%	91%	94%	96%

a) Food consumption not mentioned without explanation
b) All pups were missing on day 4 p.p.
c) All pups were found dead on day 0 p.p.

APPENDIX 3

F0 females: Food consumption and Test article intake data

Table 5.6/23 Food consumption and Test article intake data F0 females during gestation

F0 female food consumption and test article intake during **gestation** period breeding for F1 litters [1]										
	Mean absolute values				**Deviation from control[2]**			**Mean test article intake**		
	[g/animal/day]				**[%]**			**[mg/kg bw/day]**		
Day	**0 ppm**	**200 ppm**	**800 ppm**	**3200 ppm**	**200 ppm**	**800 ppm**	**3200 ppm**	**200 ppm**	**800 ppm**	**3200 ppm**
0-7	19.8	20.2	20.9	19.1	2.0	5.6	-3.5	16	67	264
7-14	20.2	20.2	20.9	19.7	0.0	3.5	-2.5	15	62	250
14-21	20.5	20.1	21	20.7	-2.0	2.4	1.0	13	53	226
Mean of means [2]:					0.0	3.8	-1.7	15	61	247

1) According to the report the food consumption data showed no test-article related changes.

2) Mean of means [%] and Deviation from control [%] are calculated from reported absolute values.

Table 5.6/24 Food consumption and Test article intake data F0 females during lactation

F0 female food consumption and test article intake during **lactation** period for nursing F1 litters [1]										
	Mean absolute values				**Deviation from control[2]**			**Mean test article intake**		
	[g/animal/day]				**[%]**			**[mg/kg bw/day]**		
Day	**0 ppm**	**200 ppm**	**800 ppm**	**3200 ppm**	**200 ppm**	**800 ppm**	**3200 ppm**	**200 ppm**	**800 ppm**	**3200 ppm**
1-4	27.1	28.0	25.5	26.8	3.3	-5.9	-1.1	22	80	356
4-7	39.2	39.6	37.8	39.0	1.0	-3.6	-0.5	29	112	483
7-14	51.0	53.6	50.2	49.3	5.1	-1.6	-3.3	38	141	579
Mean of means [2]:					3.1	-3.7	-1.7	30	111	473

1) According to report the food consumption data showed no test-article related changes.

2) Mean of means [%] and Deviation from control [%] are calculated from reported absolute values.

F1 females: Food consumption and Test article intake data

Table 5.6/25 Food consumption and Test article intake data of F1 dams during gestation

F1 female food consumption and test article intake during **gestation** period for breeding F2 litters [1]										
	Mean absolute values				**Deviation from control [2]**			**Mean test article intake**		
	[g/animal/day]				**[%]**			**[mg/kg bw/day]**		
Day	**0 ppm**	**200 ppm**	**800 ppm**	**3200 ppm**	**200 ppm**	**800 ppm**	**3200 ppm**	**200 ppm**	**800 ppm**	**3200 ppm**
0-7	20.7	19.7	20.2	19.1	-4.8	-2.4	-7.7	14	60	237
7-14	22.5	22.8	22.8	21.6	1.3	1.3	-4.0	15	63	249
14-21	24.3	23.8	23.1	23	-2.1	-4.9	-5.3	14	55	230
Mean of means [2]:					-1.9	-2.0	-5.7	14	59	239

1) According to report the food consumption data showed no test-article related changes.

2) Mean of means [%] and Deviation from control [%] are calculated from reported absolute values.

Table 5.6/26 Food consumption and Test article intake data of F1 dams during lactation

F1 female food consumption and test article intake during **lactation** period for nursing F2 litter [1]										
	Mean absolute values				**Deviation from control [2]**			**Mean test article intake**		
	[g/animal/day]				**[%]**			**[mg/kg bw/day]**		
Day	**0 ppm**	**200 ppm**	**800 ppm**	**3200 ppm**	**200 ppm**	**800 ppm**	**3200 ppm**	**200 ppm**	**800 ppm**	**3200 ppm**
1-4	31.2	31.3	32.0	30.1	0.3	2.6	-3.5	22	91	357
4-7	42.9	43.2	44.4	45.5	0.7	3.5	6.1	29	121	529
7-14	56.1	56.4	57.8	53.1	0.5	3.0	-5.3	36	152	590
Mean of means [2]:					0.5	3.0	-0.9	29	121	492

1) According to report the food consumption data showed no test-article related changes.

2) Mean of means [%] and Deviation from control [%] are calculated from reported absolute values.

5.6.2 Separate male and female studies

No data, not required

5.6.3 Three segment designs

No data, not required

5.6.4 Dominant lethal assay for the male fertility

No new studies were performed. All information available is presented in the original Annex II Dossier (1995) and has been evaluated by European authorities and Germany as Rapporteur member state.

5.6.5 Cross-matings of treated males with untreated females and vice versa

No data, not required

5.6.6 Literature - Effects on spermatogenesis

Report: II A 5.6.6/1
[redacted] 2005(a)
Effects of a low dose of Bentazon on spermatogenesis of mice exposed during foetal, postnatal and adult life
BASF DocID 2011/1257366
Date of report: 13-Jun-2005
Testing facility: not applicable
Guidelines: No test Guidelines exist for this type of study.
GLP: No, not subject to GLP regulations

Executive Summary of the Literature

Summary of published literature

The potential reproductive hazard to humans resulting from exposure to bentazone in drinking water was studied in mice. Bentazone was administration over drinking water at a concentration of 30 µg/L to: (a) 10 adult male mice (3 month old) for 100 days resulting in a dose of 21µg/kg bw and (b) 12 male mice already exposed in utero and during lactation (from 3 dams, each was allowed to nurse 4 male offsprings), and up to post natal day 100 resulting in a dose of 14 µg/kg bw. With regard to male reproductive parameters no histopathological changes were seen. Sperm number and morphology were not affected by the treatment. There were also no changes when using synaptonemal complex immunostaining or when using the micronucleus and comet assays (measures of chromosomal damage).
The only statistically significant effect seen was an alteration of the frequency of some stages of sperm maturation in both experimental compared to the concurrent control groups with no consistent pattern.
According to a review article of Creasy D. [Evaluation of Testicular toxicity in Safety Evaluation Studies: the Appropriate Use of Spermatogtenic Staging, Toxicologic Pathology, online available at http://tpx.sagepub.com/content/25/2/119] quantitating stage frequency is not appropriate as endpoint itself. This parameter is only used to identify cell loss in case of spermatid retention.
Therefore, this study is considered as supplementary information that confirms the absence of male reproductive toxicity additionally in mice at doses below those chosen for risk assessment. This Evaluation is supported by EPA in the Bentazone Registration Review Human-health Assessment from March 2010.

Classification of study: Supplementory information

5.6.7 Effects on oogenesis

No data, not required

5.6.8 Sperm motility, mobility and morphology

No data, not required

5.6.9 Investigation of hormonal activity

5.6.9.1 Peer-reviewed Literature

Report:	II A 5.6.9/1 Orton F. et al. 2009(a) Endocrine disrupting effects of herbicides and Pentachlorophenol: In vitro and in vivo evidence BASF DocID 2011/1257367
Date of report:	23-Jan-2009
Testing facility:	not applicable
Guidelines:	not applicable
GLP:	No, not subject to GLP regulations

Executive Summary of the Literature

Bentazone (purity was stated as >97 % obtained from Sigma Chemical Company Ltd.(Dorset, UK)) was tested in conjunction with 11 environmentally relevant pesticides for its endocrine disrupting potential in two in vitro assays. A recombinant yeast screen with transfected human estrogen (hER) or androgen receptor (hAR) was used to detect receptor mediated (anti-) estrogenic (YES-assay) and (anti-) androgenic activity (YAS-assay) in the concentration range 0.01-1000 µM via a colorimetric assay. Cultured Xenopus oocytes were used to measure effects on the ovulatory response and ovarian steroidogenesis in the concentration range 0.00625-62.5µM. Cytotoxicity in the respective tests was evaluated.

Bentazone induced anti-androgenic transcriptional activity in the dose range from 500 to 1000 µM in the YAS-assay after co-incubation with 2.5 nM dihydrotestosteron (DHT). An androgenic transcriptional activity in the YAS-assay was not seen.
In the YES-assay neither an agonistic nor an antagonistic potential after coincubation with 0.25 nM estradiol (E2) was observed.
No effect of bentazone was observed in the ovulation assay, which is an indication that bentazone does not affect the hormone synthesis in this test system.

An effect of bentazone as androgen antagonist in the YAS-Assay is only seen at unphysiological high concentrations. According to a publication by Bauer et al., 2002, a direct binding of bentazone to the hAR does not take place up to high concentrations. Therefore this effect seems to display an indirect effect. Anti-androgenic activity is not confirmed in any higher tier study neither in mice nor rats. Adverse effects on spermatogenesis were not observed in the carcinogenicity study in mice and reproductive performance was not affected in rats or rabbits. Therefore this finding is considered as not appropriate for hazard identification. It is considered to be not relevant for a risk assessment.

Classification of study: Supplementary information

Report:	II A 5.6.9/2 Roncaglioni A. et al. 2008(a) Binary classification models for endocrine disrupter effects mediated through the estrogen receptor BASF DocID 2011/1257368
Date of report:	20-Sep-2008
Testing facility:	not applicable
Guidelines:	not applicable
GLP:	No, not subject to GLP regulations

Executive Summary of the Literature

In this publication, which is about development of QSAR models for endocrine disruption, Bentazone is listed as part of the training set based on the Japanese METI database, which is one of the largest collections of published estrogen receptor activity databases with over 900 compounds.
The METI database contains experimentally determined values of human estrogen receptor alpha for both the receptor binding ability (RBA) and the transcriptional reporter gene activity (RA), both expressed as percentage of activity using estradiol as reference.

According to the METI database bentazone is stated to show no activity with regard to both, the human estrogen receptor alpha binding and its transcriptional activity.

Classification of study: Supplementary information

Report:	II A 5.6.9/3 Kojima H. et al. 2004(a) Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells BASF DocID 2004/1036097
Date of report:	01-Apr-2004
Testing facility:	not applicable
Guidelines:	not applicable
GLP:	No, not subject to GLP regulations

Executive Summary of the Literature

200 pesticides, including Bentazone (purity > 95%), were tested for their agonistic and antagonistic activity towards the human estrogen receptor (hER) subtypes and as well as the human androgen receptor (hAR) in transactivation assays using chinese hamster ovary (CHO) cells.
In this Luciferase reporter gene assay the pesticides were evaluated for their agonistic activity based on relative activity, expressed as that concentration that showed 20 % of the activity of 0.1 nM estradiol (E2), 1 nM E2 or 1nM dihydrotestosterone (DHT) for hER, hER or hAR, respectively. The antagonistic activity was evaluated based on the relative inhibitory activity, expressed as that concentration that showed 20 % inhibition of the activity induced by 0.01 nM E2, 0.1 nM E2 or 0.1 nM DHT for hER, hER or hAR, respectively. The pesticides were all tested up to a concentration of 10 µM to avoid cell toxicity.

Bentazone revealed neither agonistic nor antagonistic estrogen/androgen receptor activities up to concentrations of 10 µM in this in vitro Luciferase Reporter Gene Assay using Chinese Hamster Ovary cells.

Classification of study: Supplementary information

Report:	II A 5.6.9/4 Bauer E.R.S. et al. 2002(a) Development of an immuno-immobilized androgen receptor assay (IRA) and its application for the characterization of the receptor binding affinity of different pesticides BASF DocID 2011/1257371
Date of report:	01-Jan-2002
Testing facility:	n.a.
Guidelines:	n.a.
GLP:	No, not subject to GLP regulations

Executive Summary of the Literature

This article is about the development and the application of a cell free microtiter plate based receptor assay with recombinant human androgen receptor (rhAR), the so called Immuno-immobilized AR assay (IRA). This assay is claimed to exhibit identical binding characteristics as does native hAR.

Bentazone (purity 99.9% from Riedel de Haen (Seelze, Germany), dissolved in ethanol) was tested with 28 other pesticides for its ability to displace Tritium-labelled-Dihydrotestosterone (^{3}H-DHT) bound to the rhAR, which was immobilized in microtiter plates via a specific antibody. For this, a 100 µl receptor preparation aliquot in assay buffer was incubated 16 h at 4°C with 0.44 nM ^{3}H-DHT in the presence of increasing concentrations of bentazone. The receptor was fixed on the microtiter plate with a specific hAR antibody. After washing of the plates for two times with washing buffer, the specific binding of ^{3}H-DHT was measured as difference of total protein binding and non-specific binding in presence of a 200-fold surplus of unlabelled DHT.

The relative binding affinity of Bentazone to the rhAR in comparison to DHT was below 0.001% even at the highest soluble concentrate.

In summary, this study does not find any binding affinity of Bentazone to the hAR.

Classification of study: Supplementary information

Report:	II A 5.6.9/5 Bitsch N. et al. 2002(a) In vitro screening of the estrogenic activity of active components in pesticides BASF DocID 2011/1281908
Date of report:	03-Apr-2002
Testing facility:	n.a.
Guidelines:	n.a.
GLP:	No, not subject to GLP regulations

Executive Summary of the Literature

A variety of environmental chemicals were tested in vitro for their possible estrogenic activity. The E-Screen-Assay based on the human breast cancer cell line MCF-7 was used as the suitable test system. Bentazone did not show any estrogen-receptor mediated activity in this invitro test system.

Classification of study: Supplementary information

5.6.10 Teratogenicity test by the oral route in the rat

No new studies were performed. All information available is presented in the original Annex II Dossier (1995) and has been evaluated by European authorities and Germany as Rapporteur member state.

5.6.11 Teratogenicity test by the oral route in the rabbit

No new studies were performed. All information available is presented in the original Annex II Dossier (1995) and has been evaluated by European authorities and Germany as Rapporteur member state.

5.7 Neurotoxicity

Studies presented in the original Annex II Dossier (1995): No studies for neurotoxicity were submitted in the original Annex II Dossier. The results of acute, subacute/subchronic and chronic studies with bentazone in different animal species did not provide any indication of a specific neurotoxic potential of the test substance. Therefore, neurotoxicity studies were not considered necessary. It is assessed as very unlikely that bentazone would pose a specific neurotoxic hazard.

Submission of not yet peer-reviewed studies in this AIR 2-Dossier: In the "EU-MRL Dossier for the evaluation of the active substance Bentazone" a 90 day Neurotoxicity study was submitted in 2006. The summary of this study is provided in this chapter.

According to this subchronic neurotoxicity study, Bentazone did not reveal any clinical (general clinical observation, FOB and motor activity) or neurohistopathological indication of neurotoxicity.

Table 5.7/1 Summary of 90-day oral rat neurotoxicity study with Bentazone for evaluation in the AIR2 process

Test system Dose levels (ppm)	[batch /purity]	NOAEL mg/kg bw/d (ppm)	LOAEL mg/kg bw/d (ppm)	Effects at LOAEL	Reference (BASF DocID)
Wistar (CrlGlxBrlHan) rats, 0–300-1,000-3,500 ppm	(batch N 187, purity 96.9%)	M 258 F 306 (3,500)	No adverse effects	None	AIR2 Dossier IIA 5.7.4/1 [redacted] l. 2004 (b) Doc ID 2004/1013171 AIR2 Dossier IIA 5.7.4/2 [redacted] 2004 (b) Doc ID 2004/1025741

In summary, no classification is considered necessary for Bentazone as free acid, BAS 351 H, based on the neurotoxicity study. The same is considered valid for the manufactured use product Bentazone-sodium as it can be regarded as equivalent to Bentazone-acid after oral administration of equimolar doses.

Therefore, the endpoints fixed in the European Commission Review report for the active substance bentazone (7585/VI/97-final from 30 November 2000) for neurotoxicity are considered to need adaptation in regard to the new results found in the subchronic neurotoxicity study and in regard to Bentazone-sodium.

Proposal for **Endpoints for Bentazone**

Delayed neurotoxicity	No relevant endpoint
Neurotoxicity after subchronic exposure	No effects up to 3500 ppm (hdt)

5.7.1 Acute neurotoxicity - rat

In absence of any indications of neurotoxicity in the subchronic neurotoxicity study, no acute neurotoxicity study was performed. The announced study to support US regulatory needs is postponed.

5.7.2 Delayed neurotoxicity following acute exposure

Bentazone does not belong to the chemical classes suspected to cause delayed neurotoxicity. Therefore, no acute delayed neurotoxicity study was performed.

5.7.3 28-day delayed neurotoxicity

Bentazone does not belong to the chemical classes suspected to cause delayed neurotoxicity. Therefore, no 28-day delayed neurotoxicity study was performed.

5.7.4 Subchronic neurotoxicity - rat - 90-day

Report:	II A 5.7.4/1 [redacted]l. 2004(b) BAS 351 H (Bentazone) subchronic neurotoxicity study in Wistar rats - Administration in the diet for 3 months BASF DocID 2004/1013171
Date of report:	14-Apr-2004
Testing facility:	[redacted]
Guidelines:	EPA 870.6200; OECD 424
GLP:	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Report:	II A 5.7.4/2 [redacted]2004(b) Amendment No. 1: BAS 351 H (Bentazone) subchronic neurotoxicity study in Wistar rats - Administration in the diet for 3 months BASF DocID 2004/1025741
Date of report:	05-Nov-2004
Testing facility:	[redacted].
Guidelines:	EPA 870.6200; OECD 424
GLP:	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Note: This amendment corrects some errors in the final report. These consisted of the addition of an explanatory note to the equation used to calculate food efficiency and corrections regarding food and water consumption. These corrections are reflected in the summary below.

Executive Summary

Administration of Bentazone (batch N 187, purity 96.9%) to Wistar rats (CrlGlxBrlHan:Wi, Charles River, Sulzfeld, Germany) at dietary dose levels of 0, 300, 1000 and 3500 ppm for at least 91 days did not reveal any clinical (general clinical observation, FOB and motor activity) or neurohistopathological indication of neurotoxicity.

Based on the results of this study the NOEL for neurotoxicity was at least 3500 ppm, which is equivalent to about 258 mg/kg bw/d in males and 306 mg/kg bw/d in females.

(DocID 2004/1013171 & 2004/1025741)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: Bentazone (BAS 351 H)

Description:	powder / yellowish
Lot/Batch #:	N 187
Purity:	96.9%
Stability of test compound:	The test substance was stable over the study period (Expiry date 01.11.2004).

2. Vehicle and/or positive control: None

3. Test animals:

Species:	Rat
Strain:	CrlGlxBrlHan:Wi
Sex:	Male and female
Age:	49 ± 1 day at start of administration
Weight at dosing:	males: 179 ± 11.8 g; females: 142.4 ± 7.2
Source:	Charles River, Sulzfeld, Germany
Acclimation period:	at least 13 days
Diet:	Kliba maintenance diet for mouse/rats “GLP”, Provimi Kliba SA, Kaiseraugst, Switzerland, ad libitum
Water:	Tap water in bottles, ad libitum
Housing:	Single housing in DK II stainless steel wire cages (Becker & Co, Castrop-Rauxel, Germany), floor area approx. 800cm², with type 3/4 dust free bedding (SNIFF, Soest, Germany). Motor activity (MA) measurements were conducted in Polycarbonate cages with wire covers from Ehret, Emmendingen, Germany (floor area about 800 cm² and small amounts of absorbent material).
Environmental conditions:	
Temperature:	20 - 24 °C
Humidity:	30 - 70 %
Air changes:	not specified in the report
Photo period:	12 h light / 12 h dark (06:00 - 18:00 / 18:00 - 06:00)

B. STUDY DESIGN AND METHODS

1. Dates of experimental work: 25-Feb-2003 - 27-Feb-2004
(In life dates:03-Mar-2003 (first day -7 FOB and MA activity determinations) to 13-Jun-2003 (necropsy of last subset))

2. Animal assignment and treatment:

BAS 351 H was administered to groups of 10 male and 10 female rats at dietary concentrations of 0, 300, 1000 and 3500 ppm for at least 91 days. Each group was subdivided in 2 subsets (A and B) in order to balance the groups for FOB and motor activity measurements. The animals were assigned to the treatment groups by means of computer generated randomization lists based on body weights.

3. Test substance preparation and analysis:

The diets were prepared by mixing weighed amounts of test substance with a small amount of food. Subsequently, appropriate amounts of food were added to obtain the intended dietary concentrations and mixed for approx. 10 minutes in a laboratory mixer. Three diet preparations were performed for this study.

Analyses performed prior to the start of the administration period revealed that the test-substance was stable in the diet for at least 49 days when stored at room temperature.

Analysis of diet preparations for homogeneity and test-item content

Dose level [ppm]	Sampling	Analysis	Concentration[#] [ppm] Mean ± SD	Relative standard deviation [%]	Mean % of nominal concentration
0 ppm	06.03.03	07.03.03	n.d.		
	13.06.03	26.06.03	n.d.		
300 ppm	06.03.03	07.03.03	295.8 ± 6.0	2.0	98.6
	13.06.03	26.06.03	295.3 ± 0.9	0.9	98.4
average			**295.6**		**98.5**
1000 ppm	06.03.03	07.03.03	1015	-	101.5
	13.06.03	26.06.03	1028 ± 21.3	2.1	102.8
average			**1022**		**102.2**
3500 ppm	06.03.03	07.03.03	3534 ± 36.6	1.0	101.0
	13.06.03	26.06.03	3540 ± 55.9	1.6	101.1
average			**3537**		**101.0**

[#] based on mean values of the three individual samples; values may not calculate exactly due to rounding of values.
n.d.: not detectable

Homogeneity analyses of the diet preparations were performed for the low and high dose during the last pre-treatment week. For this three randomly sampled specimen of the low and high dose level were analyzed. The samples were also used for determination of the test-article concentration. A single sample was analyzed for the mid dose. An additional determination of homogeneity and test-article concentration for all dose levels was performed at the end of the administration period.

No test-article was determined in control diets.

Relative standard deviations of the homogeneity samples in the range of 0.9 to 2.1% indicate the homogenous distribution of Bentazone in the diet preparations. The actual average test-substance concentrations were in the range of 98.5 to 102.8% of the nominal concentrations.

The analyses were performed by HPLC using the following equipment/conditions:

Column:	Nucleosil 120 - 5 - C18, 250 x 4 mm
Eluent:	50% Acetonitrile + 0.5 M sulfuric acid (1000 + 5 ml)
	50% bidistilled water + 0.5 M sulfuric acid (1000 + 5 ml)
Flow rate:	1.2 ml/min
Detection:	UV, 250 nm

4. Statistics:

Means and standard deviations of each test group were calculated for several parameters. Further statistical analyses were performed according to following tables:

Statistics of clinical examinations

Parameter	Statistical test
food consumption, body weight, body weight change, food efficiency	A comparison of each group with the control group was performed using DUNNETT's test (two-sided) for the hypothesis of equal means

Statistics FOB and motor activity

Parameter	Statistical test
Feces, rearing, grip strength length forelimbs, grip strength length hind limbs, foot-splay test, motor activity	Non-parametric one- way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was performed using Wilcoxon-test. Wilcoxon-test is identical with Man Whitney-U-test (two sided) for the equal medians

Statistics of pathology

Parameter	Statistical test
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pair wise comparison of each dose group with the control group was performed using the WILCOXON test for the hypothesis of equal medians

C. Methods

1. Observations:

The animals were examined for evident signs of toxicity or mortality twice daily on working days and once daily on weekends and public holidays.

2. Body weight:

The body weight of the animals was determined before the first neurofunctional test in order to randomize the animals, at the start of the treatment (day 0), and once weekly thereafter. Additionally, body weights were also determined on the days FOB investigations were performed.

3. Food consumption, food efficiency and compound intake:

Individual food consumption was determined once a week and calculated as mean food consumption in grams per animal and day.

Food efficiency was calculated for each animal on a weekly basis based on individual values for body weight and food consumption:

$$\text{Food efficiency at day x} = \frac{BW_x - BW_y}{FC_{y\ to\ x}} \text{ x } 100$$

with BW_x and BW_y body weight [g] at day x and day y (last weighing date before day x), $FC_{y\ to\ x}$ as the mean food consumption (in g) from day y to day x, calculated as mean daily food consumption on day x multiplied with the number of days form day y to day x.

The mean daily intake of test substance (group means) was calculated based upon individual values for body weight and food consumption.

$$\text{Substance intake for day x} = \frac{FC_x \times C}{BW_x}$$

with FC_x as the mean daily food consumption (in g/day) on day x, C as the dose in ppm and BW_x as body weight on day x of the study (in g).

4. Water consumption:

Water consumption was observed daily by visual inspection of the water bottles for any overt changes in volume.

5. Ophthalmoscopy:

The eyes were examined for any changes using an ophthalmoscope prior to the start of the administration period (all animals) and towards the end of administration period (control and high dose animals).

6. Functional observation battery (FOB):

FOBs were performed in all animals prior to administration (day -7) and on study days 1, 22, 50 and 85. The FOBs were performed each time from about 10.00 a.m. onwards, started with passive observations without disturbing the animals (home cage observations), followed by removal from the home cage and open field observations in a standard arena. Thereafter, sensorimotor tests and reflex tests were conducted. The examinations were carried out by trained technicians being not aware of the group allocation of the animals. The findings were ranked according to the degree of severity, if applicable.

During the home cage observation attention was paid to posture, tremors, convulsions, abnormal movements and impairment of gait.

For open field observation the animals were transferred to a standard arena (50 x 50 cm with sides of 25 cm high) and observed for at least 2 minutes. The following parameters were assessed:

1. behavior when removed from cage
2. fur
3. skin
4. salivation
5. nose discharge
6. lacrimation
7. eyes/pupil size
8. posture
9. palpebral closure
10. respiration
11. tremors
12. convulsions
13. abnormal movements
14. impairment of gait
15. activity/arousal level
16. feces (number of fecal pellets/appearance/consistency) within two minutes
17. urine (appearance/quantity) within two minutes
18. number of rearings within two minutes

For sensorimotor tests and reflexes the animals were removed from the open field. The following tests were performed:

1. approach response
2. touch response
3. vision ("visual placing response")
4. pupillary reflex
5. pinna reflex
6. audition ("startle response")
7. coordination of movements ("righting response")
8. behavior during "handling"
9. vocalization
10. pain perception ("tail pinch")
11. grip strength of forelimbs
12. grip strength of hind limbs
13. landing foot-splay test
14. other findings

7. Motor activity measurement:

Motor activity was measured at the same day the FOB was performed in a randomized order. The measurement was performed in the dark using the Multi-Varimex-System (Columbus Instruments Int. Corp., Ohio, USA) with 4 infrared beams per cage. During the measurement the animals were kept in clean Polycarbonate cages with absorbent material. Motor activity measurements were from 2.00 a.m. onwards. The number of beam interrupts were counted over twelve 5 minute intervals. Measurement started when the first beam was interrupted by pushing the cage into the rack (staggered start). Measurements ended exactly 60 minutes thereafter. During the measurements the animals received no food and no water.

8. Hematology and clinical chemistry:

Not performed in this study.

9. Urinalysis:

Not performed in this study.

10. Sacrifice and pathology:

Five animals per sex and test group selected for neuropathology were deeply anesthetized at the end of the study and sacrificed by perfusion fixation. SOERENSEN's phosphate buffer served as rinsing solution and the fixation solution according to KARNOVSKY served as fixative.
The sacrificed animals were necropsied and the visible organs assessed by gross pathology as thoroughly as possible for perfused animals.

The weight of the brain (without olfactory bulb) was determined in all perfused animals after removal of the brain from the carcass but before any other preparation.

Various peripheral nerves, parts of the brain and brain associated organs, parts of the spinal cord and muscles were embedded and histologically examined. Details are given below:

The following organs / tissues were preserved in neutral buffered 4% formaldehyde	
✓	Brain (remaining material after trimming)
✓	Spinal cord (parts of cervical and lumbar cord)
✓	Gross lesions
The following nerves were embedded in an epoxy resin, semithin sectioned and stained with Azure II - Methylene blue basic Fuchsin and examined microscopically. (✓: all dose groups; # only control an high dose - low and mid dose organs/tissues were stored in buffer solution)	
#	Dorsal root ganglion, 3 of (C3-C6)
#	Dorsal root fiber (C3-C6)
#	Ventral root fiber (C3-C6)
#	Dorsal root ganglion, 3 of (L1-L4)
#	Dorsal root fiber (L1-L4)
#	Ventral root fiber (L1-L4)
#	Proximal sciatic nerve
#	Proximal tibial nerve (at knee)
#	Distal tibial nerve (at lower leg)
The following organ samples were embedded in paraplast, sectioned and stained with hematoxylin-eosin (H&E) (✓: all dose groups; # only control an high dose - low and mid dose organs were stored in 4% formaldehyde)	
#	Brain (cross sections): - Frontal lobe - Parietal lobe with diencephalon - Midbrain with occipital and temporal Lobe - Pons - Cerebellum - Medulla oblongata
#	Brain-associated organs/tissues - Eyes with retina and optical nerve
#	Spinal cord (cross and longitudinal sections): - Cervical swelling (C3-C6) - Lumbar swelling (L1-L4)
#	Peripheral nervous system: - Gasserian ganglia with nerve - Gastrocnemius muscle

II. RESULTS AND DISCUSSION

A. OBSERVATIONS

1. Clinical signs of toxicity

No treatment-related clinical signs were observed throughout the study.

Incidental observations included

- alopecia at various regions of body in one mid dose (1000 ppm) male and two high dose (3500 ppm) females,
- piloerection in one‡ high dose female and
- injury at the left ear in one control female.

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‡ The text section of the report indicates piloerection in two high dose female, however the summary and individual data reveal piloerection in only one high dose female.

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2. Mortality

No mortality was observed throughout the study.

3. Ophthalmoscopy

No treatment-related ophthalmoscopy findings were observed.

At termination the only findings consisted of corneal stipplings in 5 control and 3 high dose males and 2 control and 5 high dose females. The incidence of this finding was within the expected range for rats of this age.

B. BODY WEIGHT AND BODY WEIGHT GAIN

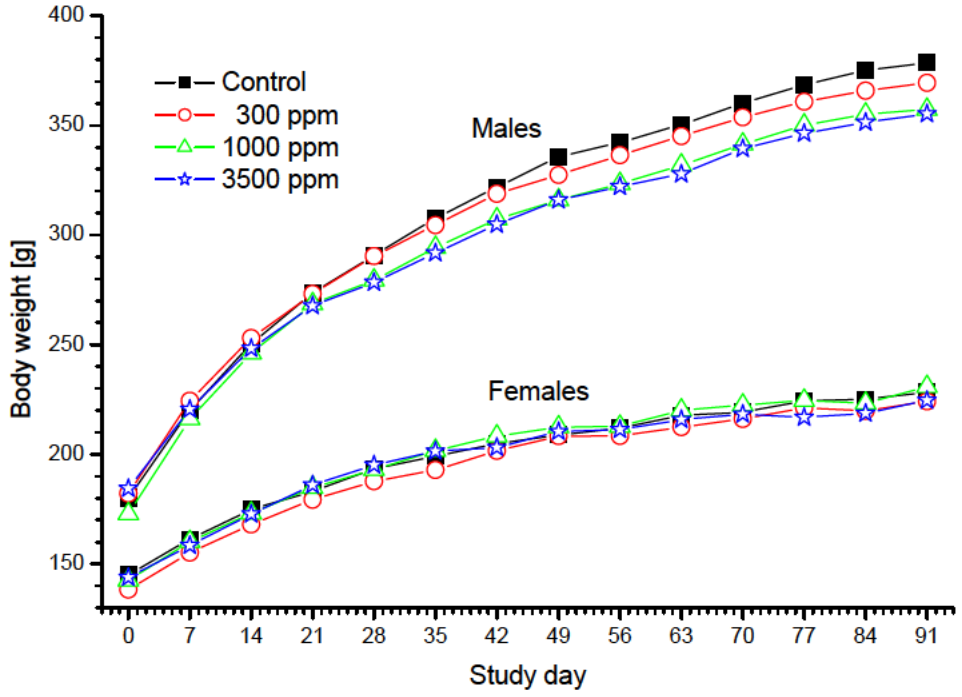
No treatment-related effects on body weight or body weight gain were observed. In absence of statistical significance the slightly lower terminal body weight and lower overall body weight gain of high dose males [see Figure 5.7/1 and Table 5.7/2] were considered to be incidental.

Table 5.7/2 Mean body weight of rats administered Bentazone for at least 91 days

	Males				Females			
Dose level [ppm]	0	300	1000	3500	0	300	1000	3500
Body weight [g]								
- Day 0	180.1	182.2	172.9	184.5	145.1	138.3	142.3	143.8
- Day 91	378.6	369.5	357.3	355.3	228.3	224.1	230.9	224.6
□% (compared to control)#		-2.4	-5.6	-6.2		-1.9	1.1	-1.6
Overall body weight gain [g]	198.5	187.3	184.4	170.8	83.2	85.8	88.4	80.8
□% (compared to control)#		-5.7	-7.1	-14.0		3.1	6.2	-2.9

Values may not calculate exactly due to rounding of mean values (values given in the table are based on unrounded means)

Figure 5.7/1 Body weight development of rats administered Bentazone for at least 91 days



C. FOOD CONSUMPTION AND COMPOUND INTAKE

A statistically significant decrease of mean daily food consumption was observed in high dose males at days 63 and 77. The isolated occurrence was not indicative of a relation to treatment.

Except of a decrease in low dose males at day 49 no statistically significant differences of food efficiency were noted in any treated group. The isolated occurrence and the lack of a dose-response indicated that the statistically significant difference in low dose males was incidental.

The mean daily test substance intake was calculated to be 21.9, 73.6 and 258.1 mg/kg in males and 27.0, 86.4 and 306.3 mg/kg in females at dietary dose levels of 300, 1000 and 3500 ppm, respectively.

D. CLINICAL PATHOLOGY

Not performed in this study.

E. FUNCTIONAL OBSERVATION BATTERY

No treatment-related FOB findings were observed at any dose level.

1. Quantitative parameters

No statistically significant differences for defecation, number of rearings, fore- and hind limb grip strength or foot splay width were observed in any treated group.

2. Home cage observations

Deviations from (rank) "zero values" were obtained in several animals [see Table 5.7/3]. However, as all findings were equally distributed between treated groups and controls. Parameters investigated included posture, tremors, convulsions, abnormal movements and impairment of gait.

Table 5.7/3 Home cage observations with deviations from rank zero (sum of 4 observations at days 1, 22, 50 and 85; 40 observations per dose level)

Sex		Males				Females			
Dose [ppm]	Rank	0	300	1000	3500	0	300	1000	3500
Posture									
- animal is sitting or lying	0	19	14	19	15	17	18	14	12
- animal is staying or moving	1	21	26	21	25	23	22	26	28
Impairment of gait									
- animal is not walking during observation	0	25	19	25	23	19	25	23	17
- no impairment of gait	1	15	21	15	17	21	15	17	23

3. Open-field observations

Deviations from "zero values" were obtained in several animals [see Table 5.7/4]. However, as all findings were equally distributed between treated groups and controls, were without a dose-response relationship or occurred in single animals only, these observations were considered incidental.

Table 5.7/4 Open field observations with deviations from rank zero (sum of 4 observations at days 1, 22, 50 and 85; 40 observations per dose level)

Sex		Males				Females			
Dose [ppm]	Rank	0	300	1000	3500	0	300	1000	3500
Behavior when removed from cage									
- animal is tense, shows no resistance against handling	0	39	40	40	40	40	40	40	40
- animal shows a slight resistance against the handling	1	1	0	0	0	0	0	0	0
Fur									
- nothing abnormal detected	0	40	40	38	40	40	40	40	35
- alopecia	4	0	0	2	0	0	0	0	5
Activity / arousal level									
- normal exploration of area	0	31	29	24	28	40	40	39	37
- reduced exploration of area	1	9	11	16	12	0	0	1	2[#]
- severe reduced exploration of area, animal apathetic	2	0	0	0	0	0	0	0	1[$]
Feces									
- no defecation during observation period	0	23	22	19	20	40	36	34	39
- feces without abnormalities	1	17	17	21	20	0	4	6	1
- soft feces	4	0	1	0	0	0	0	0	0
Urine									
- no urination during observation period	0	17	14	7	19	19	12	22	20
- urine without abnormalities	1	23	26	33	21	21	28	18	20

[#] animal 86 at days 22 and 85; [§] animal 82 at day 50

3. Sensorimotor tests / reflexes

Deviations from "zero values" were obtained in several animals [see Table 5.7/5]. However, as all findings were equally distributed between treated groups and controls, were without a dose-response relationship or occurred in single animals only, these observations were considered incidental.

Table 5.7/5 Sensorimotor activity observations with deviations from rank zero (sum of 4 observations at days 1, 22, 50 and 85; 40 observations per dose level)

Sex		Males				Females			
Dose [ppm]	Rank	0	300	1000	3500	0	300	1000	3500
approach response									
- no reaction	0	27	32	34	27	24	30	22	23
- approaching to object	1	13	8	6	13	16	10	18	16
- aggressive reaction and attacking of object	3	0	0	0	0	0	0	0	1[#]
touch response									
- no reaction	0	38	38	39	38	36	38	39	37
- orientation to the stimulus	1	2	2	1	2	4	2	1	3
behavior during handling									
- normal behavior, easy to handle, animal is tense, but it shows no resistance	0	39	40	40	40	39	39	40	40
- very easy to handle, animal is limply hanging in the hand	1	1	0	0	0	0	0	0	0
- slightly difficult to handle, animal shows a slight resistance against handling	2	0	0	0	0	1	1	0	0
vocalization									
- no or only sporadic vocalizations when touched	0	37	38	40	38	37	40	40	40
- very frequent vocalizations when touched	1	3	1	0	2	3	0	0	0
- vocalizations always when touched	2	0	1	0	0	0	0	0	0

[#] animal 82 at day 50

F. MOTOR ACTIVITY

No treatment-related changes of motor activity were noted in treated groups.

There were some statistically significant differences between control and treated groups, however these changes were neither dose related or consistent over time. Therefore, these changes were considered incidental. These changes consisted of

- increased overall activity in low dose males at day 50[‡],
- in low dose males decreased activity at interval 4 on day -7, increased activity at intervals 8 to 10 and at day 50,
- in mid dose males increased activity at interval 2 on day -7, decreased activity at interval 10 on day 22, increased activity at interval 9 and 10 on day 50,
- in high dose males increased activity at interval 2 on day -7, decreased activity at interval 7 and 10 on day 22,
- in low dose females increased activity at interval 2 and 3 at day 22, increased activity at interval 2 on day 50
- in mid dose females increased activity at interval 2 and 3 at day 22 and
- in high dose females increased activity at interval 2 at day 22.

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[‡] The text section of the report indicates a decreased overall motor activity in low dose females at day 50, however this could not be substantiated in the respective summary table (see report page 175). Instead, the mentioned increase in low dose males at day 50 is indicated (see report page 165).

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G. NECROPSY

1. Organ weight

Terminal body weights were comparable between all groups [see Table 5.7/6]. Likewise, no statistically significant differences of absolute and relative brain weights were observed.

Table 5.7/6 Mean terminal body weights and absolute and relative brain weights of rats administered Bentazone for at least 90 days

Sex		Males				Females			
Organ weight [g]	Dose [ppm]	Absolute weight [g]	□%	Relative weight [% of b.w.]	□%	Absolute weight [g]	□%	Relative weight [% of b.w.]	□%
Terminal weight [g]	0	347.82				213.1			
	300	346.68	(-0.3)			206.26	(-3.2)		
	1000	351.36	(1.0)			215.76	(1.2)		
	3500	336.56	(-3.2)			205.46	(-3.6)		
Brain	0	1.928		0.556		1.872		0.884	
	300	1.914	(-0.7)	0.554	(-0.4)	1.824	(-2.6)	0.891	(0.8)
	1000	1.964	(1.9)	0.559	(0.5)	1.802	(-3.7)	0.837	(-5.3)
	3500	1.95	(1.1)	0.581	(4.5)	1.828	(-2.4)	0.892	(0.9)

2. Gross and histopathology

No macroscopical lesions were observed at necropsy.

With the exception of axonal degeneration of lumbal ganglia in one control male no neurohistopathological lesions were noted in this study.

H. POSITIVE CONTROLS

No concurrent positive control was employed in this study.

However, in several positive control studies, behavioral and neuropathological sequelae of substances with nervous system effects were evaluated using Functional Observational Batteries (FOB), Motor Activity Measurements and Neuropathology. Clinical signs of peripheral neuropathy (e.g. ataxia, limb weakness), central neuropathy (e.g. tremors) and autonomic signs (e.g. salivation) could be shown. Histopathologically, changes in the peripheral nervous system (e.g. Wallerian-like degeneration) and central nervous system (e.g. neuronal necrosis) were seen. The motor activity device was able to show both increased and decreased activity. The inter-observer reliability of the technicians performing FOBs was proven. Thus, the ability of the methods used to detect signs of neurotoxicity was demonstrated.

Positive control studies employed single or repeated administration of the following neurotoxicants: Carbaryl, Nomifensin, Diazepam, Acrylamide and Trimethyltinchloride. Study summaries are attached to the report.

III. CONCLUSIONS

Dietary administration of Bentazone to rats at dose levels of 0, 300, 1000 and 3500 ppm did not reveal any indications of neurotoxicity.

Under the conditions of the present study the no observed effect level (NOEL) for neurotoxicity was at least 3500 ppm, which is equivalent to about 258 mg/kg bw/d in males and 306 mg/kg bw/d in females.

5.7.5 Postnatal developmental neurotoxicity

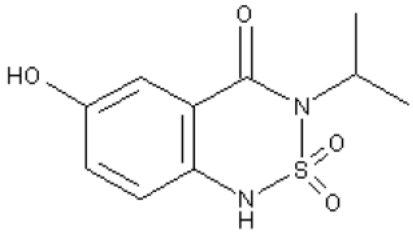
In absence of any neurotoxic potential no postnatal developmental neurotoxicity study was performed.

5.8 Toxicity studies on metabolites

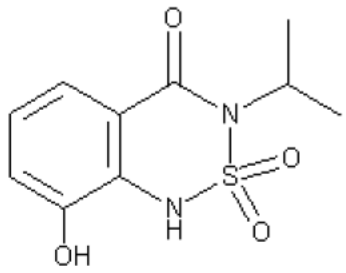
Studies presented in the original Annex II Dossier (1995): Several studies (acute and genotoxicity studies) with the plant metabolite 6-Hydroxybentazone (6-OH-Bentazone) and (acute, subchronic, developmental and genotoxicity studies) with 8-Hydroxybentazone (8-OH-Bentazone) are available, although both metabolites have been demonstrated to be formed in mammals in the low percent range (see original dossier B.5.1) and, therefore, can be regarded as included in toxicological testing of the parent compound (structures see Figure 5.8/1). These studies have been evaluated by European authorities and Germany as Rapporteur member state in 1998 (European Commission Peer Review Program, BASF Doc ID 1998/1001178) and were considered to be acceptable.

Figure 5.8/1 Chemical structures of major plant metabolites 6-OH and 8-OH-Bentazone

6-OH-Bentazone (M351H001, Reg.No. 108745)



8-OH-Bentazone (M351H002, Reg.No. 108746)



It has been shown that the 8-OH and 6-OH-metabolites of bentazone are of comparable toxicity by the oral route of administration and both less toxic then the parent compound. Additionally both metabolites were negative in the AMES assay for the potential to induce point mutations in bacteria. Since it is unlikely that a Hydroxygroup-shift in the bentazone ring system dramatically changes the toxicity, it was decided to perform more comprehensive investigations on 8-OH-Bentazone as reference substance for both metabolites. Therefore, 8-OH-Bentazone was investigated in a subchronic feeding study, in several mutagenicity studies and a prenatal developmental study. These investigations revealed that the metabolites do not have a mutagenic or teratogenic potential and are less toxic than the parent substance.

For the convenience of the reviewer, all studies submitted in the original dossier are listed separately for 6-OH-Bentazone and 8-OH-Bentazone in the following tables (Table 5.8/1 - Table 5.8/5).

6-OH-Bentazone

The acute oral studies in rats and mice revealed low acute toxicity of 6-OH-Bentazone with LD_{50} above 5,000 mg/kg bw. The exposure of Salmonella typhimurium test strains to 6-OH-Bentazone at concentrations up to and including 5,000 µg/plate did not produce an increased number of reversions, neither with nor without metabolic activation.

Table 5.8/1 Summary of peer-reviewed studies with 6-OH-Bentazone

Study type	Test system	Dose / concentr. range (batch / purity)	Result	Reference
Acute oral	Wistar rat	5,000 mg/kg bw (E-106251/ >98%)	LD_{50} > 5,000 mg/kg bw	[redacted] 1987 b Doc ID 1987/002
Acute oral	NMRI mice	1,780-3,160-5,000 mg/kg bw (E-106251/ >98%)	LD_{50} > 5,000 mg/kg bw	[redacted] 1987 c Doc ID 1987/003
Bacterial reverse mutation assay (Ames test)	*S.typhimurium* strains TA 1535, TA 1537, TA 98, TA 100; plate incorporation and pre-incubation assay; with/without S-9 mix	20-100-500-2500-5000 µg/plate (E-106251 / >98%)	negative	Engelhardt & Gelbke, 1987b Doc ID 1987/023

8-OH-Bentazone

The acute oral studies in rats and mice revealed low acute toxicity of 8-OH-Bentazone with LD50 above 5,000 mg/kg bw. The dietary subchronic study in Wistar rats revealed no observable effects up to 3,600 ppm, the highest dose tested. Thereby, 8-OH-Bentazone has shown to be less toxic than the parent compound, which had a LOEL of 1,200 ppm. Furthermore, 8-OH-Bentazone has shown no indication for a mutagenic potential in bacteria or mammalian cells as well as in an *in vivo* micronucleus test in mice.

In a prenatal developmental toxicity study, 8-OH-Bentazone did not induce maternal or developmental toxicity up to 250 mg/kg bw, which was an effect dose for the parent compound Bentazone. Additionally, there were no indications of a teratogenic potential.

Table 5.8/2 Summary of peer-reviewed acute studies with 8-OH-Bentazone

Study type	Test system	Dose / concentr. range (batch / purity)	Result	Reference
Acute oral	Wistar rat	2,150-3,830-5,000 mg/kg bw (likely L47-213/ >98.5%)	LD_{50} > 5,000 mg/kg bw	[redacted] 1987 a DocID 1987/030
Acute oral	NMRI mice	562-1,000-1,780-3,160-5,000 mg/kg bw (likely L47-213/ >98.5%)	LD_{50} > 5,000 mg/kg bw	[redacted] 1987 a Doc ID 1987/0322

Table 5.8/3 Summary of peer-reviewed 90-day oral rat study with 8-OH-Bentazone

Test system Dose levels (ppm)	[batch /purity]	NOAEL mg/kg bw/d (ppm)	LOAEL mg/kg bw/d (ppm)	Effects at LOAEL	Reference (BASF DocID)
Wistar rat, 0–400-1,200-3,600	L 47-213 / 99.9%	M 259 F 304 (3,600)	No adverse effects	None	[redacted] 1993 (a) Doc ID 1993/11011

Table 5.8/4 Summary of peer-reviewed genotoxicity studies with 8-OH-Bentazone

Study type	Test system	Dose / concentr. range (batch / purity)	Result	Reference
Bacterial reverse mutation assay (Ames test)	*S.typhimurium* strains TA 1535, TA 1537, TA 98, TA 100; plate incorporation and pre-incubation assay; with/without S-9 mix	20-100-500-2,500-5,000 µg/plate (L47-213 / 99.9%)	negative	Engelhardt & Gelbke H.-P., 1987 (a) Doc ID 1987/0168
In-vitro forward mutation assay in mammalian cells (HPRT test)	V79 cells; with/without S-9 mix	Among others 300-1,000-2,000-3,000 and 500-1,000-2,500-5,000 µg/mL (L47-213 / 99.9%)	negative	Muellerschoen H., 1992(a) Doc ID 1992/11329
In-vivo mouse micronucleus test	NMRI mouse, male and female (single oral gavage; vehicle: 0.5% aqueous CMC)	625-1,250-2,500 mg/kg bw (108746 L47-213 / 99.9%)	negative	[redacted] 1993 (a) Doc ID 1993/10424

Table 5.8/5 Summary of peer-reviewed reproduction toxicity studies with 8-OH-Bentazone

Study Dose levels (Batch / purity)	Endpoint	NOAEL mg/kg bw/d	LOAEL ppm (mg/kg bw/d)	Effects	Reference
Rat (Wistar) Prenatal developmental toxicity, **oral gavage** during days 6-15 of gestation 0-40-100-250 mg/kg bw/d (108746 L47-213/ 99.9%)	Development	250 (hdt)	-	No adverse effects	[redacted] 1993 Doc ID 1993/10572
	Maternal tox	250 (hdt)	-	No adverse effects	

hdt: highest dose tested

Submission of not yet peer-reviewed studies in this AIR2-Dossier:
Based on minor deficiencies in the original AMES test on 8-OH-Bentazone (see Monograph, Vol. 3, Annex B-5, chapter B.5.8.1.6), the test was repeated with confirmatory negative result and is given in detail within this AIR2-Dossier (see Table 5.8/6).

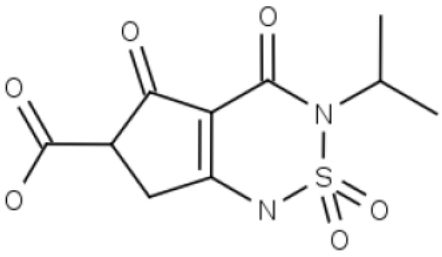
Table 5.8/6 Genotoxicity studies with 8-OH-Bentazone (Reg.No. 108746, M351H002) for evaluation in the AIR2 process

Study type	Test system	Dose / concentr. range (batch / purity)	Result	Reference
Bacterial reverse mutation assay (Ames test)	S.typhimurium strains TA 1535, TA 1537, TA 98, TA 100; E.coli strain WP2 uvrA; plate incorporation and pre-incubation assay; with/without S-9 mix	10-33-100-333-1000-2500 µg/plate in S.typhimurium and 33-100-333-1000-2500-5000 µg/plate in E.coli (L47-213/ 99,5%)	negative	AIR2 Dossier IIA 5.8/1 Woitkowiak C., 2011 DocID 2011/1123548

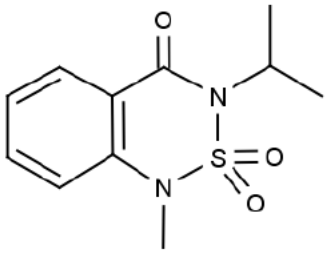
Furthermore, the **photolysis metabolites** Peak B (M351H023), Peak C (M351H024, Reg.No. 268168) and M351H025 (Reg.No. 36848) as well as the **soil- and surfacewater metabolite** N-Methylbentazone (see Figure 5.8/2) were subjected to a QSAR analysis by an external expert. The chemical structures were analysed for any indications of toxicity in the predictive computer program DEREK, which is an expert system for the identification of toxic potential from chemical structures.

Figure 5.8/2 Chemical structures of the photolysis metabolite Peak B, Peak C and M351H025 and the soil and surface-water metabolite N-Methylbentazone

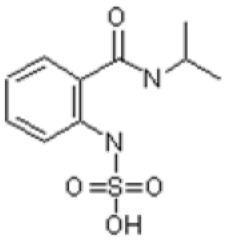
Peak B (M351H023)



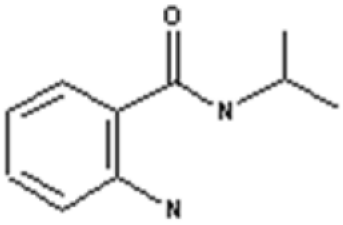
N-Methylbentazone



Peak C (M351H024, Reg.No. 268168)



M351H025 (Reg.No.36848)



Photolysis metabolites

The photolysis metabolites Peak B (M351H023), Peak C (M351H024) and M351H025 have been demonstrated to be formed in the Natural water photolysis study in the upper surface layer and are therefore regarded to be potential candidates for human exposure. The predicted environmental concentration (PEC) values were calculated to be maximal 2.8 and 1.6 µg/L for M351H023 and M351H024, and negligible for M351H025 (see AIR2-Dossier, chapter M-III A 9.8/1). Definite data requirements regarding toxicological testing of surface-water metabolites do not exist. To get indications about a possible toxicological potential, these metabolites were evaluated based on their chemical structures using DEREK.

DEREK analysis for Peak B (M351H023) revealed an alert for alpha, beta-unsaturated ketones, classified as equivocal, for chromosome damage in vitro in rodent. This alert indicates the possibility of an in vitro genotoxic alert, not relevant for bacterial cells and not relevant in vivo. If the metabolite is present at a high enough concentration in the nuclear cellular compartment, it is possible that an in vitro genotoxic response may be caused. The fact that this alert is known not to be relevant in vivo indicates that this alert has little or no significance in risk assessment.

Peak C (M351H024, Reg.No. 268168) did not reveal any alert for toxicological properties. Metabolite M351H025 (Reg.No. 36848) revealed a plausible alert for skin sensitization (and some unvalidated alerts for specific organ toxicity) which has in view of the negligible concentrations in surface water no significance in risk assessment.

N-Methylbentazone

The soil- and surface water-metabolite N-Methylbentazone has been identified as a relevant metabolite based on its biological activity in the sequential assessment performed in accordance to the "Guidance document on the assessment of the relevance of metabolites in groundwater of substances regulated under council directive 91/414/EEC" (Sanco/221/2000). As relevant metabolite it has to stay below a concentration of 0.1 µg/L. No additional screening for genotoxicity was therefore relevant. To get indications about possible toxicological potential, N-Methylbentazone has been evaluated based on its chemical structure using DEREK.
DEREK analysis revealed no alert for this structure.

In summary, the evaluation regarding plant metabolites is still valid. The investigations revealed that the plant metabolites do not have a mutagenic or teratogenic potential and are less toxic than the parent substance. Therefore a risk assessment with the parent molecule covers the risk for the plant metabolites, too. Information on photolysis metabolites does not indicate a significant risk for surface water exposure.

Therefore, the endpoint fixed in the European Commission Review report for the active substance bentazone (7585/VI/97-final from 30 November 2000) regarding other toxicological studies is considered to be still valid.

EU agreed Endpoints for bentazone (EC Review report, 2000)

Other toxicological studies	8-OH and 6-OH-metabolites, less toxic than parent compound

5.8.1 In vitro genotoxicity - Bacterial assay for gene mutation in M351H002 (Reg.No. 108 746)

Report:	II A 5.8/1 Woitkowiak C. 2011(b) Reg.No. 108 746 (metabolite of BAS 351 H, Bentazone) - Salmonella typhimurium / Escherichia coli - Reverse mutation assay BASF DocID 2011/1123548
Date of report:	30-Jun-2011
Testing facility:	BASF SE; Ludwigshafen/Rhein; Germany Fed.Rep.
Guidelines:	OECD 471 (1997); (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part B No. B.13/14; EPA 870.5100
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

S. typhimurium and E. coli were exposed to Reg.No. 108 746 (Metabolite of BAS 351 H, Bentazone) (Batch L47-213; purity 99.5%) using dimethylsulfoxide (DMSO) as a solvent in the presence and absence of metabolic activation in two independent sets of experiments. Triplicate plates were used per dose and per test condition. Vehicle and positive controls were included in each experiment.
In the plate incorporation assay Reg.No. 108 746 was tested up to the limit concentration of 5000 µg/plate. Concentrations of 33, 100, 333, 1000, 2500 and 5000 µg/plate were used with and without metabolic activation. In the preincubation test the concentrations were 10, 33, 100, 333, 1000 and 2500 µg/plate for the Salmonella strains and 33, 100, 333, 1000, 2500 and 5000 µg/plate for the E. coli strain.
In the plate incorporation assay a bacteriotoxic effect was occasionally observed depending on the strain and test conditions at concentrations of 2500 µg/plate and above. In the preincubation assay a bacteriotoxic effect was occasionally observed depending on the strain and test conditions at concentrations of 1000 µg/plate and above.
Neither in the first experiment (plate incorporation test) nor in the second experiment (preincubation test) a biologically relevant increase in the number of revertant colonies was noticed in any of the strains tested in presence or absence of metabolic activation. The positive controls induced the appropriate response in the corresponding strains, thus demonstrating the sensitivity of the test system.
According to the results of the study, the test substance Reg.No. 108 746 (Metabolite of BAS 351 H, Bentazone) is not mutagenic in the Salmonella typhimurium/ Escherichia coli reverse mutation assay under the experimental conditions of the study.
(BASF DocID 2011/1123548)

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material Reg.No. 108 746 (Metabolite of BAS 351 H, Bentazone)

Description: Solid, white

Lot/Batch #: L47-213

Purity: 99.5 % (tolerance ±1.0%)

Stability of test compound: The test substance was guaranteed to be stable until 01. May 2017, thus over the study period under the storage conditions. The homogeneity of the test substance was ensured by mixing prior to preparation of test substance formulations.
The stability of the test substance at room temperature in the vehicle DMSO and in water each over a period of 4 hours was verified.

Solvent used: DMSO

2. Control Materials:

Negative control: In parallel to each experiment, negative control experiments were carried out in order to check for possible contaminants (sterility control) and to determine the spontaneous mutation rate (vehicle control)

Vehicle control: The vehicle control with and without S-9 mix only contained the vehicle used for the test substance at the same concentration and volume for all tester strains.

Positive control compounds tested without addition of metabolic activation system:

Strain	*Mutagen*	*Solvent*	*Concentration*
TA 100	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1535	N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	DMSO	5 µg/plate
TA 1537	9-Aminoacridine (AAC)	DMSO	100 µg/plate
TA 98	4-nitro-o-phenylendiamine (NOPD)	DMSO	10 µg/plate
WP2 uvrA	4-nitroquinoline-N-oxide (4-NQO)	DMSO	5 µg/plate

Positive control compounds tested with addition of metabolic activation system:

Strain	*Mutagen*	*Solvent*	*Concentration*
TA 100	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1535	2-aminoanthracene	DMSO	2.5 µg/plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/plate
WP2 uvrA	2-aminoanthracene	DMSO	60.0 µg/plate

3. Activation:

S9 was produced from male Wistar rats. The rats were induced by daily application of 80 mg/kg b.w. phenobarbital i.p. and ß-naphthoflavone p.o. on three consecutive days and were sacrificed 24 hours after the last administration. The livers were washed with 150 mM KCL solution, minced and processed for centrifugation at 9000g. The supernatant (so called S9 fraction) was portioned and stored at -70 to -80°C.

The S9-mix was prepared freshly prior to each experiment. For this purpose, a sufficient amount of S9-fraction is thawed at room temperature and 1 volume of S9-fraction is mixed with 9 volumes of S9-supplement (cofactors). This preparation, the so-called S9-mix, was kept on ice until used.

The S9-mix was prepared immediately before use and had the following composition:

Component	*Concentration*
Sodium phosphate buffer (pH 7.4)	15 mM
Glucose 6-phosphate	5 mM
NADP	4 mM
KCl	33 mM
$MgCl_2$	8 mM
S9	10 %

4. Test organisms:

S. typhimurium strains: TA98, TA100, TA1535, TA1537

E. coli: WP2 uvrA

Salmonella typhimurium:

The Salmonella strains are checked for the following characteristics at regular intervals: deep rough character (rfa); UV sensitivity (uvrB); ampicillin resistance (R factor plasmid). E. coli WP2 uvrA is checked for UV sensitivity.

Histidine and tryptophan auxotrophy is automatically proven in each experiment via the spontaneous rate.

5. Test concentrations:

Plate incorporation assay:	Triplicate plates were prepared for each concentration (neg. control; 33, 100, 333, 1000, 2500 and 5000 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9).
Pre-incubation assay:	The test article / vehicle / positive control substance, bacterial and S-9 mix or phosphate buffer were incubated at 37°C for the duration of about 20 minutes. Triplicate plates were prepared for each concentration (neg. control; 10, 33, 100, 333, 1000 and 2500 µg/plate and positive controls at the concentrations indicated above) and condition (i.e. with and without S9) for the Salmonella strains. The respective test material concentrations for the E. coli were 33, 100, 333, 1000, 2500 and 5000 µg/plate.
Re-tests:	No re-testing was necessary.

B. TEST PERFORMANCE:

1. Dates of experimental work: 15-Mar-2011 - 14-Apr-2011

2. Plate incorporation assay:

To test tubes containing 2-mL portions of warm soft agar 0.1 mL test solution or vehicle, 0.1 mL fresh bacterial culture, 0.5 mL S9-mix (in tests with metabolic activation) or 0.5 mL phosphate buffer (in tests without metabolic activation) were added in the given order. After mixing, the samples are poured onto Vogel Bonner agar plates (minimal glucose agar plates). In the experiments with E. coli the Vogel Bonner agar plates were replaced by plates containing a SA1 selective agar according to Green and Muriel.

3. Pre-incubation assay:

100 µL of test solution or vehicle, 0.1 mL bacterial suspension and 0.5 mL S 9 mix or phosphate buffer were incubated at 37°C for about 20 minutes. Subsequently, 2 mL of soft agar was added and, after mixing, the samples are poured onto the agar plates.

After incubation in the dark for 48 to 72 hours at 37 °C each plate was checked for precipitates and status of the background lawn. Revertant colonies were counted. Means and standard deviations were calculated.

4. Titer determination:

In order to assess bacteriotoxic effects the titer was additionally determined in experimental parts with S9 mix using the vehicle control and the two highest doses. The procedure followed in both experiments (plate incorporation and pre-incubation) was the same as described above for the individual experiments with the exception that the soft agar used contained maximal amino acid solution (5 mM tryptophan or 5 mM histidine + 0.5 mM biotin). After the incubation in the dark for 48 to 72 hours at 37 °C the number of bacterial colonies was determined.

5. Statistics:

No special statistical tests were performed.

6. Evaluation criteria:

The test chemical is considered positive in this assay if the following criteria are met:

- A dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate in at least one tester strain either without S9-mix or after adding a metabolizing system.

A test substance is generally considered non-mutagenic in this test if:

- The number of revertants for all tester strains was within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.

II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test substance was stable over the study period under the storage conditions.

The stability of the test substance at room temperature in the vehicle DMSO over a period of 4 hours was verified analytically.

B. TOXICITY

A preliminary cytotoxicity assay was not performed in this laboratory. Cytotoxicity was determined in the course of the mutation assay.

Bacteriotoxic effects (slight decrease in the number of his^+ or trp^+ revertants, slight reduction in the titer) were observed in the standard plate test depending on the strain and test conditions from about 2500 µg/plate onward.

In the preincubation assay bacteriotoxicity (decrease in the number of his^+ or trp^+ revertants, reduction in the titer) was observed depending on the strain and test conditions from about 1000 µg/plate onward.

C. SOLUBILITY

No test substance precipitation was found with and without S9 mix.

D. MUTATION ASSAYS

Neither in the original nor in the confirmatory experiment with and without metabolic activation a biologically relevant increase in number of revertants was observed in any strain tested [see Table 5.8/7].

The positive controls yielded revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system.

Table 5.8/8 Bacterial gene mutation assay with Reg.No. 108 746 - Mean number of revertants

Strain	TA 98		TA 100		TA 1535		TA 1537		E. coli	
Experiment 1 (original): Plate incorporation assay										
Metabol. activation	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Neg. control (DMSO)	27	28	101	108	17	16	8	7	29	41
Reg.No. 88691										
33 µg/plate	23	28	105	114	14	15	7	8	35	47
100 µg/plate	22	32	105	112	18	16	7	8	32	45
333 µg/plate	27	28	108	102	16	17	9	9	31	41
1000 µg/plate	23	19	94	115	15	18	7	8	32	42
2500 µg/plate	12B	11B	91B	82B	15B	12B	5B	6B	29	31
5000 µg/plate	0B	0B	0B	0B	0B	0B	0B	0B	21B	17B
Pos. control[§]	454	568	703	650	934	165	404	170	724	238
Experiment 2 (confirmatory): Preincubation assay										
Metabol. activation	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Neg.control (DMSO)	26	27	98	105	14	14	7	8	34	45
Reg.No. 88691										
10 µg/plate	28	28	98	99	14	16	5	7		
33 µg/plate	21	28	94	106	13	16	7	7	33	48
100 µg/plate	21	27	95	100	13	16	6	8	41	40
333 µg/plate	22	24	84	101	17	13	7	8	40	39
1000 µg/plate	15	27	80	85	13	10	5	6	36	45
2500 µg/plate	0B	0B	0B	0B	0B	0B	0B	0B	30	33
5000 µg/plate									0B	6B
Pos. control[§]	552	571	737	813	755	133	401	126	573	241

[§] = Compound and concentrations see Material and Methods (I.A.2.) above
B = reduced background growth

III. CONCLUSIONS

According to the results of the present study, the test substance Reg.No. 108 746 (Metabolite of BAS 351 H, Bentazone) is not mutagenic in the Salmonella typhimurium/Escherichia coli reverse mutation assay under the experimental conditions chosen here.

5.8.2 QSAR Analysis of Photolysis metabolites and N-Methylbentazone

Note: The QSAR Analysis on the Photolysis metabolites and N-Methylbentazone contains additionally confidential information on impurities. Therefore the report is located in the confidential part (see AIR2-dossier Document JM2 A 5.8/1 Doc ID 2011/1128248). To maintain the data protection of the confidential part the QSAR report is not given in the public part of the AIR2 dossier, but the relevant data are summarized in here.

Executive Summary of the QSAR Analysis (Report Doc ID 2011/1128248 is located in the Confidential part JM2)

Four metabolites are candidates for potential human exposure via ground- or surface water. The photolysis metabolites of Bentazone Peak B (M351H023), Peak C (M351H024, Reg.No. 268168) and M351H025 (Reg.No. 36848) have been demonstrated to be formed in the Natural water photolysis study in the upper surface layer. Definite data requirements regarding toxicological testing of surface-water metabolites do not exist. No studies were performed.
Additionally, the soil- and surface water-metabolite N-Methylbentazone has been identified as a relevant metabolite based on its biological activity in the sequential assessment performed in accordance to the "Guidance document on the assessment of the relevance of metabolites in groundwater of substances regulated under council directive 91/414/EEC" (Sanco/221/2000). As relevant metabolite it has to stay below a concentration of 0.1 µg/L, which is fulfilled. No additional screening for genotoxicity was necessary.

To get indications about possible toxicological potential, the metabolites have been evaluated by an external DEREK expert with more than 25 years experience in this activity, based on their chemical structure using DEREK Nexus, a knowledge base expert system for the prediction of toxicological hazard based on an analysis of chemical structure.

Based on this QSAR analysis the **photolysis metabolite Peak B (M351H023)** was identified to have an alert for Chromosome damage in vitro, classified as equivocal. This alert indicates the possibility of an in vitro genotoxic alert, not relevant for bacterial cells and not relevant in vivo. The fact that this alert is known not to be relevant in vivo indicates that this alert has little or no significance for risk assessment. **Peak C (M351H024, Reg.No. 268168)** was identified to have no alert. **M351H025 (Reg.No. 36848)** was identified to have a plausible alert for skin sensitization which is in view of the expected negligible concentrations in surface water not relevant for risk assessment. For the soil- and surface water-metabolite N-Methylbentazone the DEREK-analysis revealed no alert.

(BASF Doc ID 2011/1128248)

I. MATERIAL AND METHODS

1. Test Material

The structures of the test items were provided by the sponsor and are given in Figure 5.8/2.

2. Description of the DEREK system

Each structure was run through the most recent version of the DEREK software and rulebase (Program Version Derek Nexus 2.0.0.201011301322 and Knowledge base version 11_05_09_2011, last modified at 14 March 2011).
DEREK Nexus is a knowledge base expert system for the prediction of any indications of toxicity based on an analysis of chemical structure. DEREK Nexus uses a knowledge base, which covers a wide variety of important toxicological end points, which include carcinogenicity, mutagenicity, skin sensitisation, reproductive toxicity, irritation, and respiratory sensitisation. The DEREK Nexus knowledge base is written and maintained by experts and is one of the recommended and accepted QSAR Databases by regulatory authorities (for reference see "Guidance Document On The Assessment Of The Equivalence Of Technical Materials Of Substances Regulated Under Council Directive 91/414/EEC" (Sanco/10597/2003 - rev. 9, 17 June 2011)). A list of publications, which give details of the development of the system, and some details of how the system is used are attached in the Original Report in the Reference section. Authors of these publications include the scientists who developed the original concept of the program, and an article by an US EPA expert in structure-activity.
Further details of the system can be found on the supplier's web site http://www.lhasalimited.org/ under the section for DEREK. The supplier of DEREK, Lhasa Ltd., is a not-for-profit organisation.

3. Expert evaluation

Based on the DEREK database generated structural alerts an expert evaluation was made for each molecule in the context of the significance of the alert in risks for toxicity and taking into account the parent molecule alerts. The evaluations were based on over 25 years experience of using the DEREK system, and over 30 years in toxicology.

4. Validation and Probability of alerts

The alerts are divided into fully expert committee-validated alerts and not fully expert committee-validated Rapid Prototype rules (RP). Both types of alerts are classified for their probability into 9 classes (certain, probable, plausible, equivocal, doubted, improbable, impossible, open, contradicted)

B. TEST PERFORMANCE:

1. Dates of work: Draft report: 10-Nov-2011
Final report 29-Nov-2011

2. Endpoints covered: The knowledge base covers a wide variety of important toxicological end points, which include carcinogenicity, mutagenicity, skin sensitisation, reproductive toxicity, irritation, and respiratory sensitisation. The species covered in the knowledge base are bacterium, Escherichia coli, Salmonella typhimurium and all mammalian species. It is well known that the physicochemical properties of a compound play an important role in determining potential toxicity. *DEREK Nexus* calculates Log Kp (by the Potts & Guy equation) Log P (by the Moriguchi estimation) and Molecular weight (by LPS). These values are used in the DEREK assessment where appropriate (e.g. when skin penetration is a factor in assessing the significance of a finding).
These data are not given in this dossier but are available in the original report.

II. RESULTS AND DISCUSSION

In the following the results and the discussion of the QSAR analysis are given.

Peak B metabolite (M351H023)

This photolysis metabolite of Bentazone had alert 309, classed as equivocal, for alpha, beta-unsaturated ketone, chromosome damage in vitro. This alert indicates the possibility of an in vitro genotoxic alert, not relevant for bacterial cells and not relevant in vivo. If the metabolite is present at a high enough concentration in the nuclear cellular compartment, it is possible that an in vitro genotoxic response may be caused. The fact that this alert is known not to be relevant in vivo indicates that this alert has little or no significance in risk assessment.

Peak C metabolite (M351H024, Reg No.268168)

This photolysis metabolite of Bentazone revealed no alert.

Metabolite M351H025 (Reg.No. 36848)

This photolysis metabolite of Bentazone had alert 427, classed as plausible so that the weight of evidence supports the proposition, for aromatic primary or secondary amine: "Skin sensitisation in mammal". If present in the epidermis at sufficiently high concentrations, this metabolite could cause a sensitisation reaction. Additionally, M351H025 had alert RP*152 for Aniline or precursor, splenotoxicity and alert RP*154 Aniline or precursor, bone marrow toxicity, both classified as equivocal so that there exists an equal weight of evidence for and against the proposition. These alerts are not fully validated by the expert committee and show a low degree of probability and are therefore per se regarded as to have a minor predictivity. Furthermore M351H025 was shown to have negligible environmental concentrations in surface water so that it is not considered to represent any risk.
In addition to the DEREK analysis, in the internal BASF database, two acute oral rat studies exist [1973/0056, 1983/098] demonstrating an LD_{50} in rats (Wistar) of 2840 mg/kg bw. A dermal irritation/corrosion study in rabbits has shown no indication of an irritant property to the skin [1995/11403]. M351H025 was tested negative in an AMES [1997/1000241].

N-Methylbentazone had no alerts identified.

III. CONCLUSIONS

The DEREK structure-activity hazard identification revealed for the photolysis metabolite Peak B (M351H023) an alert for in vitro chromosome damaging property, classed as equivocal. This alert indicates the possibility of an in vitro genotoxic alert, not relevant for bacterial cells and not relevant in vivo, so that it has little or no significance for risk assessment. Further toxicological investigations were not performed. Peak C (M351H024) revealed no alert. The photolysis-metabolite M351H025 revealed a plausible alert for sensitization but represents in view of the negligible concentrations expected in the surface water no risk. N-Methylbentazone revealed no alert.

5.9 Medical and clinical data

The medical data were updated within the course of the literature search, which is documented in detail with regard to the used search profile and strategy in the Literature Search Report (Doc ID 2012/1007281, for summary of results in the area of mammalian toxicology see Excel file Doc ID 2012/1007283).

5.9.1 Report on medical surveillance on manufacturing plant personnel

All persons handling crop protection products are surveyed by regular medical examinations. There are no specific parameters available for effect monitoring of bentazone. Thus, the medical monitoring programme is designed as a general health check-up, with special interest in the primary target organs presumed to be relevant by analogy from animal experiments.

The surveillance program includes a general physical examination including neurological status, red and white blood cell counts, and liver enzymes. Adverse health effects suspected to be related to bentazone exposure have not been observed.

Some cases of irritation of the eyes and the skin have been registered in the BASF-internal clinical incident log in persons exposed to bentazone.
No other adverse health effects due to bentazone have been documented in the BASF-internal medical files.

The literature search revealed the following publication:

Report:	II A 5.9.1/1 Nasterlack M. et al. 2006(a) Epidemiological and clinical investigations among employees in a former herbicide production process BASF DocID 2011/1281903
Date of report:	13-Jul-2006
Testing facility:	not applicable
Guidelines:	not applicable
GLP:	No, not subject to GLP regulations

Executive Summary of Literature

At BASF sites in Germany and US studies have been performed among employees, which had been assigned to bentazone production facilities in the 1970s and 1980s. However, these studies do not address potential effects of the final product, but those of a specific multi-step batch process that included a large number of process starting materials and chemical intermediates and that is now obsolete.

(DocID 2011/1281903)

5.9.2 Report on clinical cases and poisoning incidents - Literature

The literature search retrieved several case reports of suicide attempts with bentazone, some of which resulted in deaths. Additionally, there are several unpublished reports of deaths after ingestion of bentazone. The lowest reported dose associated with a death was 20 g; however, this information is related to a case, which occurred in China, and could not be verified.

In general, the published cases of human intoxication confirm the toxicological profile of bentazone found in animals, with the blood, kidney and the liver as target organ. The clinical signs after ingestion of Bentazone in humans regarding respiratory problems, vomiting, diarrhoea, muscle rigidity and abdominal pain are in line with the findings in acute toxicity studies. The changed blood parameters for creatinine, BUN and blood coagulation were also observed in animal studies. Lethality occurred between 2 hours and 5 days after ingestion at dose levels comparable to those found to be lethal in animals. Nevertheless based on the inaccuracy of the data and the confounding factors like health status or for example the detoxification measures it is difficult to interpret the data in regard to comparability of humans and animals susceptibility. Under consideration of the reported and verifiable cases here, there is no indication that humans are more sensitive to Bentazone than animals.

Report:	II A 5.9.2/1 Wu I-W. et al. 2007(a) Acute renal failure induced by Bentazone: 2 case reports and a comprehensive review BASF DocID 2011/1281900
Date of report:	24-Feb-2007
Testing facility:	not applicable
Guidelines:	not applicable
GLP:	No, not subject to GLP regulations

Executive Summary of Literature

A 23-year-old healthy male farmer attempted to commit suicide by consumption of approximately 80 mL of bentazone (35.3 g, 569 mg/kg bw). He developed nausea, vomiting, cough, abdominal pain, nasogastric irrigation and received a gastric lavage. The patient developed a stable recovery and was discharged 5 days after admission.
Another 31-year-old man, suffering from alcohol abuse and schizophrenia, had ingested approximately 200 mL bentazone (88.2 g; 1,764 mg/kg bw). Icteric sclera, multiple reddish ulcers in the oral cavity, tongue base and posterior wall of the oropharynx were found. In the next days the patient developed acute renal failure, fluid overloading and high anion gap metabolic acidosis; and he died five days after admission.

(DocID 2011/1281900)

Report:	II A 5.9.2/2 Emre H. et al. 2010(a) Rhabdomyolysis and acute renal failure as a result of Bentazone intoxication BASF DocID 2011/1281901
Date of report:	01-Jan-2011
Testing facility:	not applicable
Guidelines:	not applicable
GLP:	No, not subject to GLP regulations

Executive Summary of Literature

After ingestion of approximately 36 g bentazone for suicidal attempt a 41-year-old man developed rhabdomyolysis with acute renal failure, vomiting, palpitation, fever and somnolence. 15 hours after intake, urine output decreased, and blood urine nitrogen, creatinine and creatinine kinase levels were increased and prothrombin time was increased. On the fifth day of hospitalization, laboratory findings had turned to normal levels.

(DocID 2011/1281901)

Report:	II A 5.9.2/3 Mueller I.B. et al. 2003(a) Case report - Fatal overdose of the herbicide Bentazone BASF DocID 2011/1281902
Date of report:	10-Apr-2003
Testing facility:	not applicable
Guidelines:	not applicable
GLP:	No, not subject to GLP regulations

Executive Summary of Literature

A 59-year-old woman who intentionally ingested 100-200 ml Basagran (about 50-100 g bentazone) was taken to the hospital with a cardiac arrest 2 days after she had consumed the herbicide. During this period she suffered vomiting, urination and diarrhoea and she was drowsy with a muddled speech. Biological samples obtained at the autopsy were analysed and presence of bentazone, alcohol and an active metabolite of citalopram (antidepressant) were detected. Blood concentrations of bentazone, alcohol and desmethyl-citalopram were 625 mg/kg, 0.62 g/l and 0.03 mg/kg, respectively.

(DocID 2011/1281902)

Report:	II A 5.9.2/4 Turcant A. et al. 2003(a) Case report - Fatal acute poisoning by Bentazon BASF DocID 2011/1281904
Date of report:	01-Mar-2003
Testing facility:	not applicable
Guidelines:	not applicable
GLP:	No, not subject to GLP regulations

Executive Summary of Literature

A case of fatal suicidal bentazone poisoning is presented along with a description of the different analytical methods involved. A 56-year-old farmer was examined by the family doctor 1 h after voluntarily ingesting 500 mL of FIGHTER (about 250 g bentazone). He presented a Glasgow score of 15, polypnea, diarrhea, and vomiting. During transport by ambulance to the hospital, he tossed, sweated, and suddenly presented breathing difficulty followed by heart failure. The patient died within 2 h postingestion. Blood and urine samples were taken just before death. Bentazone plasma and urine levels were 1500 and 1000 mg/L, respectively.

(DocID 2011/1281904)

Note: The following literature is mentioned in Emre H. et al. 2011(a) (DocID 2011/1281901) as reference and was, based on the publication year, not integral part of the literature search. Nevertheless it is regarded as relevant and therefore summarized here.

Report:	II A 5.9.2/5 Lin T.-J. 1999(a) Case report - Acute Basagran poisoning mimicking neuroleptic malignant syndrome BASF DocID 2011/1281905
Date of report:	09-Mar-1999
Testing facility:	not applicable
Guidelines:	not applicable
GLP:	No, not subject to GLP regulations

Executive Summary of Literature

A 27-year-old robust man, without any medical and surgical history, attempted to commit suicide by consumption of 300 ml basagran (about 130 g bentazone). This poisoning resulted in vomiting, fever, sweating, pipe-like muscle rigidity, sinus tachycardia, drowsiness, leukocytosis, rhabdomyolysis and hepatorenal damage. Emperical treatment with bromocriptine was temporally associated with resolution of above signs and symptoms. His clinical presentations and the effect of bromocriptine may be indicative that basagran poisoning mimicks neuroleptic malignant syndrome.

(DocID 2011/1281905)

5.9.3 Observations on general population exposure & epidemiological studies

Neither data on exposure of the general public nor epidemiologic studies are available for BASF SE, nor is BASF SE aware on any epidemiologic studies performed by third parties.

5.9.4 Clinical signs and symptoms of poisoning and details of clinical tests

All data known regarding clinical signs and symptoms of poisoning are reported under point 5.9.2.

5.9.5 First aid measures

See safety data sheet / precautions; symptomatic and supportive treatment.

5.9.6 Therapeutic regimes

No specific antidote is known; symptomatic treatment is recommended, therefore.

5.9.7 Expected effects & duration of poisoning as a function of exposure

Expected effects were derived for acute and subacute studies in animals. The information in humans are limited to the case reports given under chapter 5.9.2. Specific signs of toxicity or specific clinical test methods are not known.

5.9.8 Effects & duration of poisoning as a function of time

Expected effects were derived for acute and subacute studies in animals. The information in humans are limited to the case reports given under chapter 5.9.2.
Specific signs of toxicity or specific clinical test methods are not known.

5.9.9 Dermal penetration

Studies presented in the original Annex II Dossier (1995): The dermal penetration of Bentazone was tested as sodium salt in aqueous solution in vivo in the rat (see Monograph, Vol.3, Annex B-5, September 1996, chapter 5.1.1.5, BASF DocID 1985/299) and revealed a low bioavailability of less than 2% of total dose. The study has been considered to be in compliance with Directive 87/304/EEC requirements of that time. The study has been evaluated by European authorities and Germany as Rapporteur member state in 1998 (European Commission Peer Review Program, BASF Doc ID 1998/1001178) and was considered to be acceptable.

Submission of not yet peer-reviewed studies in this AIR2-Dossier:
No new studies were submitted in the AIR 2 Document M-II. A new in vitro dermal penetration study in human skin with the representative bentazone SL-type solo-formulation confirms the low dermal penetration of bentazone, which is shown to be at a maximum of 1.3 % in human skin. A detailed description of the study is given in the Document M-III A 7.6.

5.10 Other/special studies

Studies presented in the original Annex II Dossier (1995): Bentazone was tested with regard to its general pharmacological and EEG effects. The studies were conducted according to the JMAFF No 59 NohSan No 4200 guideline-requirements. The studies have been evaluated by European authorities and Germany as Rapporteur member state in 1998 (European Commission Peer Review Program, BASF Doc ID 1998/1001178) and were considered to be supplementary as these kind of studies are not required for EU registration. However, it was agreed that these studies provide additional information. For the convenience of the reviewer, these studies are listed in Table 5.10/1.

Table 5.10/1 Summary of peer-reviewed special toxicity studies with Bentazone

Study Dose levels (Batch / purity)	Pharmaological Effects	Reference (BASF DocID)
Rat (Wistar), Study on EEG effects, gavage 0-400-500-700-1000 mg/kg bw (N187 / 97.8%)	No pathological EEG change detectable	[redacted] 1986 Doc ID 1986/427 [redacted] 1987 DocID 1987/062
Rat (Wistar male, KFM/Han), Mice (NMRI male, KFM), Rabbits(New Zealand White male, KFM) Guinea Pigs (Himalayan White spotted male) General pharmacology study (N187 / 97.8%)	Bentazone at very high dose levels may have slight effects on the autonomic nervous system. A depression effect on the arterial blood pressure seen in rats was not reproducible (see list of studies not submitted, Doc ID 2010/1173295)	[redacted]. 1987 Doc ID 1987/0132

Submission of not yet peer-reviewed studies in this AIR 2-Dossier: An acute neurotoxicity study in rats and a 28 day Immunotoxicity study in mice with Bentazone-acid were announced in the Application for Approval. These studies are not mandatory in the EU but in the US registration process. As the US registration process is at a later date, the studies have been postponed and no data are available to be submitted in the AIR2 process. In view of the properties of Bentazone the missing of these studies is not regarded as critical; it does not present a data gap.

A mechanistic study regarding the renal transporter involved in Bentazone excretion in female rats has been performed and is given in detail in the Bentazone AIR 2 Dossier Document M-II 5.1.1/3, Doc ID 2011/1265806). This study has revealed that Bentazone is excreted in female rats via the saturable Organic Anion Transporter.

Furthermore several publications were found in the external literature search (see Literature Search Report Doc ID 2012/1007281, for summary of results see Excel file Doc ID 2012/1007283) about special tests performed with Bentazone regarding nuclear cell receptor agonistic properties, for example. The publications are given in detail under point 5.10.1. Shortly summarized, Bentazone is reported to show no effect on metabolic activity of phagocytes but seem to induce a reduction in leukocyte migration ability in vitro. Furthermore it was shown in vitro that Bentazone does not activate or interact with the Aryl-Hydrocarbon Receptor, the Peroxisome proliferator-activated receptors α or γ, or the human and mouse pregnane X receptor. This is in line with the findings in toxicological studies which show no indication for nuclear receptor mediated enzyme induction.

5.10.1 Peer-reviewed Literature

Report:	II A 5.10/1 Pistl J. et al. 2003(a) Determination of the immunotoxic potential of pesticides on functional activity of sheep leukocytes in vitro BASF DocID 2011/1257370
Date of report:	27-Jan-2003
Testing facility:	not applicable
Guidelines:	not applicable
GLP:	No, not subject to GLP regulations

Executive Summary of Literature

Bentazone (purity stated as 99.9%, supplier Sigma-Aldrich, USA) was tested for its effect on the function of phagocytes and lymphocytes isolated from the peripheral blood of sheep under in vitro conditions. The blood samples were from 5 healthy Merino sheep. Leukocytes were isolated from the peripheral blood by the method of osmotic shock of erythrocytes. Bentazone was dissolved in dimethylsulfoxide at concentrations of 10^{-1} to 10^{-6} M as stock solution, and was thereafter used to constitute a 1% maintenance medium with the final concentrations between 1000 µM to 0.01 µM.
The tests performed were:

a) The Iodo-nitro-tetrazolium (INT) reductase test, in which the metabolic activity of phagocytes during phagocytosis is spectrophotometrically quantified at 485 nm by means of formazan-generation out of INT. A metabolic activity ratio index of phagocytic cells (activated by starch) vs basic metabolism both under bentazone treatment was derived.
b) The leukocyte migration-inhibition assay (LMIA), in which a Migration Index (MI) as the ratio of leukocyte migration after T-Lymphocyte stimulation via phytohemagglutinine versus the migration without stimulation is determined. An immunotoxic effect is characterized in this assay as a decreased ability of lymphocytes to respond to the mitogenic stimuli of phytohemagglutinine, which is calculated as ratio of MI pesticide / MI control. MI pesticide is the ration of migration in the presence of the pesticide with versus without PHA. MI control is the ration of migration in presence of 1% DMSO with versus without PHA. A cytotoxic effect is characterized by a decreased migration ability of leukocytes in presence of the pesticide versus that in presence of 1 % DMSO.

Bentazone showed no effect on the metabolic activity of phagocytes but revealed a cytotoxic effect at 1mM and a significant reduction of T-Lymphocyte activated migration of leukocytes in the concentration range between 1 – 100 µM. According to the author this in vitro results are in line with a 3-month subchronic intoxication of sheep with Bentazone (1/10 and 1/20 LD_{50}) which revealed a decrease in the response of lymphocytes to mitogenic activation from the eighth week of the experiment but no effect of this pesticide on phagocytic activity (Mikula et al., 1992, Immune response of organism at subchronic intoxication with herbicide bentazon, TP. Vet. Hum. Toxicol. 34, 507-9).

Because the immune system is highly complex the relevance of in vitro test results is less strong than in vivo data. Based on the routine toxicity testing parameters (hematology, organ weights and histopathology) the immune system was not identified as specific target organ neither in rodents nor in non rodent regulatory toxicity studies.

Report:	II A 5.10/2 Takeuchi S. et al. 2008(a) In vitro screening for aryl hydrocarbon receptor agonistic activity in 200 pesticides using a highly sensitive reporter cell line, DR-EcoScreen cells, and in vivo mouse liver cytochrome P450-1A induction by Propanil, Diuron and Linuron BASF DocID 2011/1262291
Date of report:	05-Oct-2008
Testing facility:	not applicable
Guidelines:	not applicable
GLP:	No, not subject to GLP regulations

Executive Summary of Literature

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor that regulates genes involved in xenobiotic metabolism, cellular proliferation and differentiation. In this study, a highly sensitive AhR-mediated reporter cell line, DR-EcoScreen cells, which are mouse hepatoma Hepa1c1c7 cells stably transfected with a reporter plasmid containing seven copies of dioxin-responsive element has been developed. Using these DR-EcoScreen cells, reporter gene assays were performed and the AhR agonistic activities of 200 pesticides (29 organochlorines, 11 diphenyl ethers, 56 organophosphorus pesticides, 12 pyrethroids, 22 carbamates, 12 acid amides, 7 triazines, 6 ureas, and 45 others) has been characterized, beyond them Bentazone.

Bentazone has shown to be not active as AhR agonist. This is in line with the findings in toxicological studies.

Report:	II A 5.10/3 Takeuchi S. et al. 2006(a) In vitro screening of 200 pesticides for agonistic activity via mouse peroxisome proliferator-activated receptor (PPAR)alpha and PPARgamma and quantitative analysis of in vivo induction pathway BASF DocID 2011/1278450
Date of report:	06-Sep-2006
Testing facility:	not applicable
Guidelines:	not applicable
GLP:	No, not subject to GLP regulations

Executive Summary of Literature

Peroxisome proliferator-activated receptors (PPARs) are ligand-dependent transcription factors and key regulators of lipid metabolism and cell differentiation. However, there have been few studies reporting on a variety of environmental chemicals, which may interact with these receptors. In the present study, the mouse PPARα and PPARγ agonistic activities of 200 pesticides (29 organochlorines, 11 diphenyl ethers, 56 organophosphorus pesticides, 12 pyrethroids, 22 carbamates, 11 acid amides, 7 triazines, 8 ureas and 44 others) by in vitro reporter gene assays using CV-1 monkey kidney cells have been characterized, beyond them Bentazone.
Bentazone has been shown to activate neither PPARα- nor PPARγ in these in vitro assays. This is in line with the findings in toxicological studies.

Report:	II A 5.10/4 Kojima H. et al. 2010(a) Comparative study of human and mouse pregnane X receptor agonistic activity in 200 pesticides using in vitro reporter gene assays BASF DocID 2011/1278451
Date of report:	27-Nov-2010
Testing facility:	not applicable
Guidelines:	not applicable
GLP:	No, not subject to GLP regulations

Executive Summary of Literature

The nuclear receptor, pregnane X receptor (PXR), is a ligand-dependent transcription factor that regulates genes involved in xenobiotic metabolism. Recent studies have shown that PXR activation may affect energy metabolism as well as the endocrine and immune systems. In this study, we characterized and compared the agonistic activities of a variety of pesticides against human PXR (hPXR) and mouse PXR (mPXR). We tested the hPXR and mPXR agonistic activity of 200 pesticides (29 organochlorines, 11 diphenyl ethers, 56 organophosphorus pesticides, 12 pyrethroids, 22 carbamates, 12 acid amides, 7 triazines, 7 ureas, and 44 others) by reporter gene assays using COS-7 simian kidney cells, beyond them Bentazone.

Bentazone has been shown to elicit neither hPXR- nor the mPXR-receptor mediated transcriptional activity in these in vitro assays. This is in line with the findings in toxicological studies.

5.11 Summary of mammalian toxicity and overall evaluation

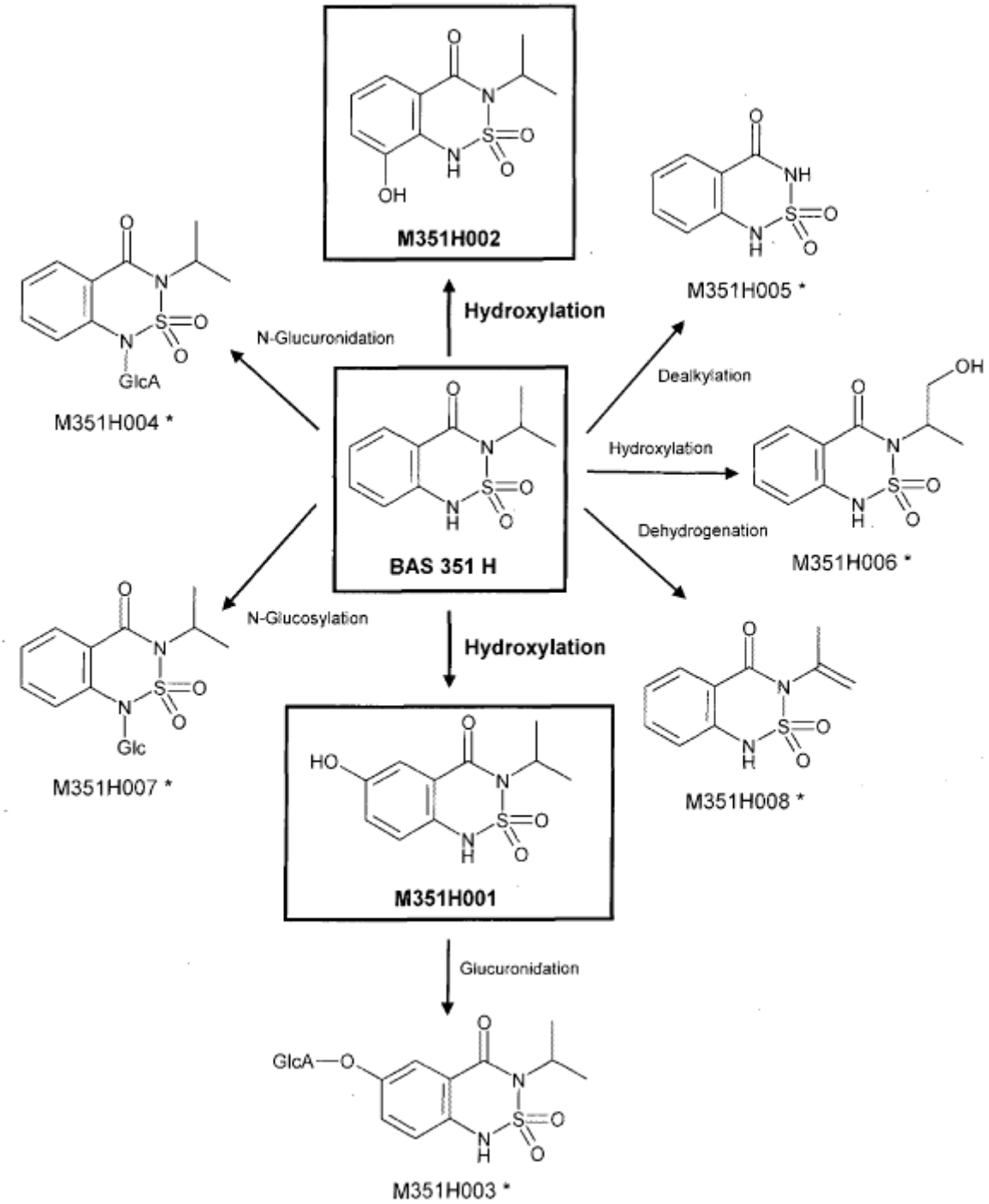
A. TOXICOKINETICS AND METABOLISM

A series of studies has been performed on rats, mice and rabbits to determine the kinetics of bentazone. The metabolic pathway of bentazone was elucidated in these species following oral (rat and rabbit) or intravenous administration (mouse) (see Figure 5.11/1).

Studies on toxicokinetics have shown that bentazone is rapidly absorbed resulting in a high bioavailability of about 90% following oral administration. In contrast, dermal absorption in rats is rather low with less than 2% of total dose. A new in vitro study with a bentazone SL-type solo-formulation demonstrated that dermal penetration through human skin will not exceed 1.3 %.

Elimination is rapid with urine being the major route of excretion (approximately 90% of the dose). Only traces (1% to 2 %) were found in the feces and in the bile. Repeated administration did not significantly alter the pattern of absorption and elimination. There was no evidence for accumulation of bentazone. Nevertheless, the area under the plasma radioactivity curve (AUC) per unit dose for rats revealed significantly higher values for the high dose groups compared to the low dose groups. It was possible to show that the non-linear region in disposition is based on saturation of excretion via the Organic anion transporter and that saturation of excretion starts between actual dose levels of 84.7 and 165.9 mg/kg bw (calculated as bentazone sodium salt) in female rats after bolus administration.
Beside this observation, all species investigated (rat, rabbit. mouse, goat and hen) revealed considerable similarities in kinetic studies.
Bentazone was only poorly metabolized with the parent compound being the predominant excretion product. Only small amounts of the metabolites M351H001 (6-0H-bentazone) and M351H002 (8-0H-bentazone) could be detected in animals. Further metabolites, which were present only in trace amounts, resulted from glucuronidation of metabolite M351H001 (M351H003), N-glucuronidation (M351H004), dealkylation (M351H005), hydroxylation (M351H006), N-glucosylation (M351H007) and dehydrogenation (M351H008) of the parent compound.

Figure 5.11/1 Proposed metabolic scheme of bentazone in the rat



* Metabolites were identified by HPLC-MS. Identification and quantitation with HPLC was not feasible due to negligible amounts.

B. ACUTE TOXICITY

The **most current studies** to assess the acute health effects of **BAS 351 H** are listed in Table 5.11/1. Based on these representative studies, BAS 351 H, administered as free acid, is characterized by a moderate acute oral toxicity and low dermal and inhalation toxicity in rats. Unspecific signs of systemic toxicity (if at all) were observed in acute oral, dermal or inhalation studies. The sodium salt revealed similar toxic properties. Bentazone-acid is not a skin irritant but a moderate eye irritant. Furthermore bentazone is a skin sensitizer; however, dilutions of bentazone-sodium of 10 % and below induced no sensitization in guinea pig. Bentazone is not phototoxic.

Table 5.11/1 Summary of the core acute and photo-toxicity studies with Bentazone

Type of study	Test substance	Result classification	Reference
Oral route - rat (batch / 93.9%)	BAS 351 H (acid)	LD50 = 1,470 mg/kg bw EU classification R22 GHS classification H302	Monograph B 5.2.1.1 [redacted], 1983 DocID 1983/114
Oral route - rat (Bentazone tech.)	BAS 351 H (sodium salt)	LD50 = 1,480 mg/kg bw (sodium salt) equivalent to LD 50 = 1,356 mg/kg bw (free acid) EU classification R22 GHS classification H302	Monograph B 5.2.1.4 [redacted] 1973 DocID 1973/023
Percutaneous route - rat (270 778 / 94.6%)	BAS 351 H (acid)	LD50 > 5,000 mg/kg bw EU classification not required GHS classification not required	Monograph B 5.2.2.2 [redacted] 1978 DocID 1978/055
Inhalation route - rat (N187 / 97.8%)	BAS 351 H (acid)	LC50 > 5.1 mg/l EU classification not required GHS classification not required	Monograph B 5.2.3.1 [redacted] 1986 DocID 1986/220
Skin irritation - rabbit (Bentazone tech.)	BAS 351 H (acid)	Not irritating to skin EU classification not required GHS classification not required	Monograph B 5.2.4.1 [redacted] 1983 DocID 1983/081
Eye irritation - rabbit (Bentazone tech.)	BAS 351 H (acid)	Irritating to eye EU classification R36 GHS classification H319	Monograph B 5.2.5.1 [redacted] 1983 DocID 1983/083
Skin sensitization - Maximization Test (MS 1 F 22/ 94 %)	BAS 351 H (acid)	Sensitizing EU classification R43 GHS classification H317	Monograph B 5.2.6.1 [redacted], 1986 DocID 1986/195
Skin sensitization – Open Epicutaneous Test (WH 4976 / 60 %)	BAS 351 H (sodium salt)	Sensitizing EU classification R43 GHS classification H317	Monograph B5.2.6.2 [redacted], 1986 DocID 1986/221&1986/347
NRU-Phototoxicity study Balb/c 3T3 cells clone A31 (COD-001416 / 100%)	BAS 351 H (acid)	Not phototoxic EU classification not required GHS classification not required	AIR2-Dossier II A 5.2.7/1 Cetto V.& Landsiedel R., 2011 (a); DocID 2011/1110261

C. SHORT-TERM TOXICITY

Short-term toxicity studies (28 - 90 days) with oral administration of bentazone-acid are available from three different species (rats, mice, dogs). In addition, a comparative 90-day study is available demonstrating the equivalence of bentazone-acid and bentazone-sodium. Further studies performed with Bentazone-acid are a one-year oral dog study and three 21-day dermal toxicity studies in rabbits. Table 5.11/2 provides a listing of these studies including information on NOAELs and LOAELs as well as the effects observed at the respective LOAELs.

Predominant target after short-term to subchronic oral exposure of rats, mice and dogs was the blood coagulation system (prolonged blood coagulation time and subsequent haemorrhages at higher dose levels). Changes in clinical chemistry (without clear pattern) in combination with relative liver weight changes as well as increased water consumption and partly higher urinary output combined with increased kidney weights suggested that the kidney and the liver are additional target organs, although there were no histopathological changes at any dose level. Body weight changes were induced in all species at higher dose levels or after prolonged administration. The comparison of the NOELs has shown that interspecies variation of bentazone is rather low.

Short-term dermal administration of bentazone up to 1,000 mg/kg bw did not reveal any adverse effects in two valid studies in rabbits. A third one confirmed this result but was considered unacceptable due to coccidia infection.

Table 5.11/2 Subacute and subchronic toxicity of BAS 351 H

Type of study Dose levels (Batch/Purity)		NO(A)EL mg/kg bw/d (ppm)	LOAEL mg/kg bw/d (ppm)	Effects at LOAEL	Reference
Supplementary Study: 28-day dietary Rat (Fischer 344) Bentazone (acid) 0-600-1800-4000*-10000 ppm (batch unknown/93.9%)	M	196 (1800)	554 (4000*)	Hemorrhages in kidneys	Monograph B 5.3.1.1 [redacted] 1981 DocID 1981/10240
	F	217 (1800)	607 (4000*)	Hemorrhages in ovaries	
Supplementary Study: 28-day dietary Mouse (B6C3F1) Bentazone techn. (acid) 0-400-2000-5000-10000ppm (N 169 / 93.9%)	M	Setting not justified	407 (2000)	Prolonged blood clotting	Monograph B 5.3.1.2 Anonymous, 1981 DocID 1981/10239 [redacted] 1982 DocID 1982/1000611
	F	Setting not justified	487 (2000)		
Supplementary Study: 90-day dietary Rat (Sprague-Dawley) Bentazone techn. (acid) 0-70-200-800-1600 ppm (batch / purity unknown)	M F	~10 (200)	(800)	Kidney weight increase	Monograph B 5.3.2.1 [redacted] 1970 DocID 1970/008
90-day dietary Rat (Wistar, KFM (Han) BAS 351 H (acid) 0-400-1200-3600 ppm (N 187, 97.8 %)	M	25.3 (400)	77.8 (1200)	Clinical chemistry changes	Monograph B 5.3.2.2 [redacted] 1987 DocID 1987/0173
	F	29** (400)	86.1** (1200)	BW gain reduced (6%) Clinical chemistry changes Urinalysis changes Kidney weight increase	
90-day dietary Rat (Wistar, Crl:Wi (Han)) BAS 351 H (Reg.No.51929) (COD-001416/100%) 0-3600 ppm	M	n.a.	238 (3600)	Prolonged blood clotting	AIR2-Dossier II A 5.3.2/1&2 [redacted] 2011 & 2012 DocID 2011/1173365 DocID 2012/1009658
	F	n.a.	252 (3600)	Increased water consumption Clinical chemistry changes Kidney weight increase (rel./abs.) Liver weight increase (rel.)	
90-day dietary Rat (Wistar, Crl:Wi (Han)) BAS 351 H-Na (Reg.No.88691) (COD-001417/91.9%) 0-475-1425-4275 ppm equivalent to 0-400-1200-3600 ppm calculated as Bentazone-acid	M	76† (1200)	244† (3600)	Prolonged blood clotting Kidney weight increase (rel.)	
	F	82† (1200)	256† (3600)	Increased water consumption Clinical chemistry changes Kidney weight increase (rel./abs.) Liver weight increase (rel.)	
90-day dietary Beagle dog Bentazone techn. (acid) 0-100-300-1000-3000 ppm (batch / purity unknown)	M & F	12 (300)	39.6 (1000)	Clinical findings	Monograph B 5.3.2.3 [redacted] 1970 DocID 1970/009 DocID 1973/0057 DocID 1972/0061
1-year dietary Beagle dog Bentazone techn. (acid) 0-100-400-1600 ppm (N 187 / 97.8%)	M	13.1 (400)	49.7 (1600)	BW reduction Anemia, bloody diarrhea Prolonged blood clotting	Monograph B 5.3.2.4 [redacted] 1989 DocID 1989/0049 DocID 1989/0153
	F	13.2 (400)	54.8 (1600)	BW reduction Anemia, bloody diarrhea Prolonged blood clotting	

Table 5.11/2 Subacute and subchronic toxicity of BAS 351 H

Type of study Dose levels (Batch/Purity)	NO(A)EL mg/kg bw/d (ppm)	LOAEL mg/kg bw/d (ppm)	Effects at LOAEL	Reference
21-day dermal toxicity – rabbit (New Zealand) Bentazone techn. 0; 250; 500; 1,000 mg/kg bw (batch / purity unknown)	M & F 1000	not identified	no adverse findings	Monograph B 5.3.3.3 [redacted] 1971 DocID 1971/005
21 day dermal toxicity – rabbit (New Zealand) BAS 351 H (acid) 0; 250; 500; 1,000 mg/kg bw (N 187 / 97,8%)	M & F -	-	Excluded from evaluation due to infection	Monograph B 5.3.3.2 [redacted] 1988 DocID 1988/0350
21-day dermal toxicity – rabbit (New Zealand) BAS 351 H (acid) 0; 250; 500; 1,000 mg/kg bw (N 194 / 97.64%)	M & F 1000	not identified	no adverse findings	Monograph B 5.3.3.1 [redacted] 1993 DocID 1993/10760

* nominal 5000 ppm corresponded to an actual concentration of about 4,000 ppm
† Substance intake in mg/kg bw and ppm is given as Bentazone acid equivalent
** According to the monograph the NOAEL was set at 400 ppm for males and females, however females show effects one dose group higher.
n.a. not applicable

D. GENOTOXICITY STUDIES

Bentazone was investigated for its mutagenic properties in various studies - recently in a complete set of regulatory relevant in vitro and in vivo studies with the manufacturing use product bentazone-sodium according to nowadays guideline requirements (see Table 5.11/3) - with all three endpoints of genetic damage (point or gene mutation, chromosome mutation and DNA damage and repair) being covered. A summary of the older studies already evaluated in the former EU registration process is given below in Table 5.11/4

Based on the most current studies performed on Bentazone-sodium, it can be summarized that bentazone does not induce point mutations in bacterial or mammalian cells in the presence or absence of metabolic activation. The result of a chromosome aberration study *in vitro* was positive for clastogenic properties with and without S9 but negative for aneugenic effects. This study fits to a positive *in vitro* chromosome aberration study listed in the Monograph. Therefore it is considered that bentazone might possess clastogenic activity *in vitro*.

The test for chromosomal aberration *in vivo* was negative indicating the lack of a clastogenic potential of bentazone in mammals. This is supported by the older studies performed with Bentazone-acid, including studies on germ-cells. Additionally no increase in DNA-synthesis was observed after bentazone-sodium treatment in Wistar rats.

Considering the weight of evidence, the assessment can be made that bentazone does not have a mutagenic or genotoxic property in vivo.

Table 5.11/3 Not peer reviewed genotoxicity studies with Bentazone-sodium for evaluation in the AIR 2 process

Study type	Test system	Dose / concentr. range* (batch / purity)	Result	Reference (BASF DocID)
Bacterial reverse mutation assay (Ames test)	S.typhimurium strains TA 1535, TA 1537, TA 98, TA 100; E.coli strain WP2 uvrA; plate incorporation and pre-incubation assay; with/without S-9 mix	33-100-333-1000-2750-5500 µg/plate (COD-001417 / 91.9%)	negative	AIR2-Dossier II A 5.4.1/1 Woitkowiak, 2011 DocID 2011/1106427
In-vitro chromosome aberration assay in mammalian cells	V79 cells; with/without S-9 mix	187.5-375-750-1500-3000 µg/mL (COD-001417 / 91.9%)	positive for clastogenicity negative for aneugenicity with and without S9	AIR2-Dossier II A 5.4.2/1 Schulz & Landsiedel, 2011 DocID 2011/1106426
In-vitro forward mutation assay in mammalian cells (HPRT test)	CHO cells; with/without S-9 mix	187.5-375-750-1500-3000 µg/mL without S9 187.5-375-750-1500-3000 and 536-1071-2143-3000 µg/mL with S9 (COD-001417 / 91.9%)	negative	AIR2-Dossier II A 5.4.3/1 Schulz & Landsiedel, 2011 Doc ID 2011/1184494
In-vivo micronucleus test	NMRI mouse, ♂ & ♀, single oral (gavage) application; vehicle: Deionized water)	♂: 340-680-1360 mg/kg bw ♂:217.5-435-870 mg/kg bw (COD-001417 / 91.9%)	negative	AIR2-Dossier II A 5.4.4/1 [redacted] 2011 (c) Doc ID 2011/1184970 [redacted] 2011 (e) Doc ID 2011/1277971
In-vivo Unscheduled DNA Synthesis (UDS)	Wistar rat, ♂ (single oral gavage); analysis of rat hepatocytes prepared 3 or 14 h after exposure	0-275-550-1100 mg/kg bw (COD-001417 / 91.9%)	negative	AIR2-Dossier II A 5.4.5/1 [redacted] 2011 (d) Doc ID 2011/1192857 AIR2-Dossier II A 5.4.5/2 [redacted] 2011 (f) DocID 2011/1277972
Published Literature: Wing spot test on somatic mutation and recombination	Drosophila melanogaster, standard and high bioactivation fly crosses,	0.05 mM - 5 mM (not reported/>95%)	negative in standard, positive in high bioactivation fly cross	AIR2-Dossier II A 5.4.7/1 Kaya B. et al. 2003 (a) DocID 2011/1257369

* Dose or concentration range is given for the test substance bentazone-sodium, thereby about 10% higher than the concentration of the active ingredient bentazone-acid

Table 5.11/4 Summary of Genotoxicity studies with Bentazone

Study type	Test system	Substance Dose / concentr. range (batch / purity)	Result a) without b) with S9-mix	Reference (BASF DocID)
Bacterial reverse mutation assay (Ames test)	*S.typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100; *E.coli* strain WP2 hcr	Bentazone 0-10-50-100-500-1,000-µg/plate (batch not given/ 94%)	a) negative b) negative	Monograph B 5.4.1.1.6 Monograph B 5.4.2.1 Shirasu Y et al., 1976 Doc ID 1976/009
Bacterial reverse mutation assay (Ames test)	*S.typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100; *E.coli* strain WP2 hcr	Bentazone 0-1,000-2,500-5,000-10,000 µg/plate (batch not given/ 94%)	a) negative b) negative	Monograph B 5.4.1.1.7 Moriya M, 1984 Doc ID 1984/10285 Addendum to 1976/009
Bacterial reverse mutation assay (Ames test)	*S.typhimurium* strains TA 1537, TA 98, TA 100;	Bentazone (77/357) 0-3.1-10-31-100-310-1,000-2,000 µg/plate (batch not given/ 92.5%)	a) negative b) negative	Monograph B 5.4.1.1.1 Oesch F. 1977 Doc ID 1977/028
Bacterial reverse mutation assay (Ames test)	*S.typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100;	Bentazone (83/3) 0-20-100-500-2,500-5,000 µg/mL (batch not given/ 96.7%)	a) negative b) negative	Monograph B 5.4.1.1.2 Gelbke &.Engelhardt, 1983 Doc ID 1983/222
Bacterial reverse mutation assay (Ames test & E.coli reverse mutation assay)	*S.typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100; *E.coli* strain WP2 uvrA	Bentazone 0-20-100-500-2,500-5,000 µg/mL (N169 / 92.6%%)	a) negative b) negative	Monograph B 5.4.1.1.3 Gelbke &.Engelhardt, 1985 Doc ID 1985/108
Bacterial reverse mutation assay (Ames test)	*S.typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100;	Bentazone-sodium (pure and technical grade) 0-500-1,000-2,500- 5,000-7,500-10,000 µg/mL (84/298/ not given; 84/299/ 550g/l)	a) negative b) negative	Monograph B 5.4.1.1.4 Gelbke &.Engelhardt, 1985 Doc ID 1985/081
Published literature: Bacterial reverse mutation assay (Ames test)	*S.typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100; *E.coli* strain WP2 hcr	Bentazone Up to 5,000 µg/mL (batch&purity not given)	a) negative	Monograph B 5.4.1.1.8 Moriya M et al., 1983a Doc ID 1983/10177
Published literature: Bacterial reverse mutation assay (Ames test)	*S.typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100; *E.coli* strain WP2 hcr	Bentazone (not given / 99.9%)	a) negative b) negative	Monograph B 5.4.1.1.8 Jeang C.-L., Li G.-C., 1978 Doc ID 1978/10236 Doc ID 1980/10294
Published literature: Bacterial reverse mutation assay (Ames test & E.coli reverse mutation assay)	*S.typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, TA 100; *E.coli* strain WP2 hcr	Bentazone (batch&purity not given)	a) negative b) negative	Monograph B 5.4.1.1.8 Shirasu Y et al., 1982a; Doc ID 1982/10230
Published literature: DNA damage and repair (SOS Chromotest)	*E.coli*	Bentazone (batch&purity not given)	a) negative b) negative	Monograph B 5.4.1.1.9 Xu H.H & Schurr K.M., 1990 Doc ID 1990/10692
Published literature: DNA damage and repair (Mitotic gene conversion assay)	*Saccharomyces cerevisiae* strain D4	Bentazone (batch&purity not given)	a) negative	Monograph B 5.4.1.2.1 Siebert D.,Lemperle E., 1974 Doc ID 1974/10200
Published literature: DNA damage and repair (Mitotic gene conversion assay)	*Saccharomyces cerevisiae* strain D4	Bentazone (batch&purity not given)	a) negative	Monograph B 5.4.1.2.1 Zimmermann F.K. et al., 1984 a Doc ID 1984/10261

Table 5.11/4 Summary of Genotoxicity studies with Bentazone

Study type	Test system	Substance Dose / concentr. range (batch / purity)	Result a) without b) with S9-mix	Reference (BASF DocID)
In-vitro chromosome aberration assay in mammalian cells	CHO cells	Bentazone a) 0-500-1000-2,000-3,000 µg/ml b) 0-2,000-3,000-4,000-5,000 µg/ml (batch&purity not given)	a) negative b) negative	Monograph B 5.4.1.3.5 Taalman & Hoorn, 1987 Doc ID 1987/0169
In-vitro forward mutation assay in mammalian cells (HPRT test)	CHO cells	Bentazone techn. (84/140) 0-100-464-1,000-2,150-4,640-10,000 µg/ml (N 169/ 93.9%)	a) negative b) negative with rat S9-mix c) weakly positive with mice S9-mix	Monograph B 5.4.1.3.1 Gelbke & Jaeckh, 1985 Doc ID 1985/396
In-vitro forward mutation assay in mammalian cells (HPRT test)	CHO cells	Bentazone techn. (84/140) 1,250-2,500-5,000-7,500-10,000-12,500-15,000 µg/ml (batch not given/ 93.9%)	a) negative b) negative with rat S9-mix c) negative with mice S9-mix	Monograph B 5.4.1.3.2 Boer W.C., den, 1985 a Doc ID 1985/403
In-vitro forward mutation assay in mammalian cells (HPRT test)	CHO cells	Bentazone techn. 0-100-600-1,200-2,500-5,000 µg/ml (N194/ 97.6%)	a) negative b) negative	Monograph B 5.4.1.3.3 Muellerschoen & Völkner, 1991 Doc ID 1991/11108
Chromosome analysis in vivo (Micronucleus test)	NMRI Mouse	Bentazone techn. 0-200-400-800 mg/kg bw single application (MS2F22/ 95.6%)	negative	Monograph B 5.4.2.4 [redacted] 1985 Doc ID 1985/036
Published literature: Chromosome analysis in vivo (Chromosome analysis and Micronucleus test)	Wistar rats	Bentazone techn. 0-27,5-55-110-220-700 mg/kg bw Two oral administrations with 24 h time lag (batch&purity not given)	negative	Monograph B 5.4.2.5 Postica F. et al., 1982 Doc ID 1982/10236
In-vivo Unscheduled DNA Synthesis (UDS)	B6C3F1 mice	Bentazone techn. (84/140) 40-360 mg/kg bw single application	negative	Monograph B 5.4.2.6 [redacted] 1985 Doc ID 1985/159
In-vivo mutation assay in germ cells (Dominant lethal test in vivo	Rat (Sprague-Dawley)	Bentazone Dietary concentration: 20-60-180 ppm over 13 weeks (batch&purity not given)	negative	Monograph B 5.4.2 [redacted] 1971 Doc ID 1971/018
In-vivo mutation assay in germ cells (Dominant lethal test in vivo	Mouse (NMRI)	Bentazone techn. Single intraperitoneal application: 195 mg/kg bw (batch&purity not given)	negative	Monograph B 5.4.2.2 [redacted] 1973 Doc ID 1973/025
In-vitro Unscheduled DNA Synthesis (UDS)	Mouse Primary hepatocytes (B6C3F1)	Bentazone (84/110) 0.05 up to 1004 µg/ml in WME medium	negative	Monograph B 5.4.1.3.7 Cifone M.A. & McKeon M, 1985 Doc ID 1985/067

E. CHRONIC TOXICITY AND CARCINOGENICITY

The chronic toxicity and potential carcinogenicity of bentazone was tested in rats and mice. The results of these studies are summarized in Table 5.11/5

Table 5.11/5 Summary of long term studies performed with Bentazone

Study Dose levels (Batch / purity)	Sex	NOAEL ppm (mg/kg bw/d)	LOAEL ppm (mg/kg bw/d)	Effects at LOAEL and above	Reference (BASF DocID)
Rat (Sprague-Dawley), 2-year (oral) chronic toxicity, diet M & F: 0-100-350-1,600 ppm (batch & purity not given)	M & F	350 (17)	1,600 (76)	Reduced body weight and food consumption; increased organ weights	Monograph, B 5.5.1 [redacted] 1974 Doc ID 1974/004
Rat (Fischer 344, Du/Crj (SPF)), 2-year (oral) combined chronic toxicity / carcinogenicity, diet M & F: 0-200-800-4,000 ppm (N 169; 93.9%)	M F	200 (9) 200 (11)	800 (35) 800 (45)	Impaired blood coagulation, impaired liver and kidney function indicated by changes in clinical chemistry and organ weights. At 4,000 ppm reduced body weight	Monograph, B 5.5.2 [redacted] 1985 Doc ID 1985/433 [redacted] 1985(a) Doc ID 1985/440 [redacted] 1986(a) Doc ID 1986/0438 [redacted] 1989(a) Doc ID 1989/10485 [redacted] 1988 (a) Doc ID 1988/0155
Mice (Swiss Webster) 18 month oral carcinogenicity study, diet M & F: 0-100-350-1,600 ppm (batch & purity not given)	M & F	350 (52)	1,600 (237)	High mortality (>50%) in test and control groups. At 1,600 ppm reduced food consumption and body weight, organ weight changes.	Monograph, B 5.5.3 [redacted] 1974 Doc ID 1974/041
Mice (CFLP) 82-95 week oral carcinogenicity, diet M & F: 0-100-350-1,600 ppm (p.195.75 / purity not given)	M F	1600 (138.4) 1600 (152.8)	– –	No adverse effects	Monograph, B 5.5.4 [redacted] 1978 Doc ID 1978/034
Mouse (B6C3F1) 2-year (oral) combined chronic toxicity/carcinogenicity, diet M & F: 0-100-400-2,000 ppm (N 169; 93.9%)	M F	100 (12)	400 (47)	Impaired blood coagulation, increased testicular calcification (questionable effect, not confirmed in other studies); proliferative lesions in the liver (females) At 2,000 ppm transient reduction of body weight gain (males), hemorrhages in liver and heart	Monograph B 5.5.5 [redacted] 1985 Doc ID 1985/432 [redacted] 1985 Doc ID 1985/431 [redacted] 1987 Doc ID 1987/0139 [redacted] 1988 Doc ID 1988/0483 [redacted] 1987 Doc ID 1987/10417

Long term treatment in rats resulted in an impairment of body weight gain in both sexes at 4,000 ppm and a transient decrease in both sexes at 800 ppm. Feed consumption was not regularly influenced, but water consumption was clearly increased in the 4,000 ppm animals and transiently also in the 800 ppm group. Accordingly was the urine volume increased and specific gravity decreased. Some changes in hematological and clinical chemistry parameters in the 4,000 and 800 ppm groups indicated an effect on the kidneys in the 6 and 12-month, but were not seen at 24 months. Blood coagulation effects were seen at 800 ppm (47 mg/kg bw) in males and one male died at 4,000 ppm due to hemorrhagic lesions. Kidney (and other organ) weight changes were most prominent after 6 months, but faded out in the later phases.
Neither gross nor histopathology revealed lesions that could be attributed to the administration of bentazone. Lesions of eye ball and atrophy of the optic nerves were observed in association with cataract in male animals of the 4,000 ppm group at month 24 and were assessed as age-induced manifestations in this strain of rats. Tumors and age-related changes noted in many rats which died from month 13 onwards or were sacrificed at the end of the study were assessed to be not substance-related. In particular, there were no findings indicating an oncogenic potential. Furthermore, the number of tumors, tumor bearing animals and number tumors per animal was not influenced by bentazone. Thus no oncogenic potential was found in this study.

Dietary administration of Bentazone to mice for 2 years resulted in a transient impairment of body weight development in males at 2,000 ppm. Bentazone caused further a prolongation of the prothrombin time in the males at 400 and 2,000 ppm which is in agreement with similar findings in the short-term toxicity studies in mice and in rats. The gross-pathological examination showed various lesions in the liver, spleen and thymus in animals of all the treatment groups and the control group. These lesions occurred in some cases significantly more frequently only in the two intermediated dose groups and showed no dose-response relationship. They were assessed as being age-induced and not being related to the test-substance. Testis histology revealed an increased incidence of calcification of the testicular tunica albuginea and deferent canals in the 400 and 2,000 ppm males. In view of historical control data, this change is a very common lesion in aged mice including the strain used in this study. There was in addition no evidence of another adverse effect of bentazone on testes or on male reproductive performance obtained in any study on bentazone. There were no neoplastic changes indicative of an effect of Bentazone.

Regarding all information available from the chronic studies, the overall conclusion was drawn that bentazone has no carcinogenic potential but impairs primary the blood coagulation.

The more recent combined chronic toxicity / carcinogenicity study in rats ([redacted] 1985, Doc ID 1985/433) served as basis for calculation of the ADI with a NOAEL of 200 ppm, equivalent to 10 mg/kg bw (combined sexes) .

F. REPRODUCTION TOXICITY

The prenatal toxicity of bentazone was determined in both rats and rabbits. The reproduction toxicity of the test substance was investigated in two multigeneration studies in rats. Results are summarized in Table 5.11/6. In addition, a reevaluation of the most current multigeneration study has been performed resulting in changes regarding the NOAEL for maternal toxicity and the corresponding substance intake (changed values are indicated in the table by bold italian style). These changes are important for the evaluation of dependency of offspring toxicity, but does not change the fixed endpoints set in the monograph on Bentazone.

Table 5.11/6 Summary of reproduction toxicity studies with BAS 351 H

Study Dose levels (Batch / purity)	Endpoint	NOAEL ppm (mg/kg bw/d)	LOAEL ppm (mg/kg bw/d)	Effects at LOAEL	Reference DocID
Rat (Sprague-Dawley), Multigeneration (3-gen) study, diet 0-20-60-180 ppm (batch / purity not given)	Fertility	180 (18, hdt)	–	No evidence of impaired fertility	Monograph, B 5.6.1.1. [redacted] 1973 Doc ID 1973/010
	Offspring toxicity	180 (18, hdt)	–	No offspring toxicity	
	Parental toxicity	180 (18, hdt)	–	No parental toxicity	
Rat (Wistar/HAN), Multigeneration (2-gen) study, diet 0-200-800-3,200 ppm (N187 / 97.8%)	*Fertility* changed in comparison to monograph (1996)	*3,200* *(230)* Not derived	–	No evidence of impaired fertility	Monograph, B 5.6.1.2 [redacted] 1989 Doc ID 1989/0068 AIR 2-Dossier II A 5.6.1/3 - 5 [redacted] 2011 Doc ID 2011/1248852 [redacted] 2011 Doc ID 2011/1145234 [redacted] 2009 Doc ID 2011/1262290
	Offspring toxicity changed in comparison to monograph (1996)	200 ***(22)*** 200 ~~(14)~~	800 ***(80)*** 800 ~~(56)~~	Reduced pup body weight secondary to maternal toxicity	
	Parental toxicity changed in comparison to monograph (1996)	***200*** ***(22)*** ~~800~~ ~~(56)~~	***800*** ***(80)*** ~~3,200~~ ~~(247)~~	Reduced food consumption and body weight of F0 dams at PND 1-4	
Rat (Sprague-Dawley) Prenatal developmental toxicity, **oral gavage** during days 6-15 of gestation 0-22.2-66.7-200 mg/kg bw/d (batch/purity not available)	Development	- (66.7)	- (200)	Elevated resorption rate, reduced bw, evidence of teratogenicity (increased number of runts, anasarca) *Remark: Teratogenic effects not reproducible* (see II A 5.6.10/2 1978/039)	Monograph B 5.6.2.4 [redacted] (1971) Doc ID 1971/0041
	Maternal tox.	- (200, hdt)	-	No adverse effects	
Rat (Sprague-Dawley) Prenatal developmental	Development	- (200, hdt)	-	No adverse effects	Monograph B 5.6.2.5 [redacted]

Table 5.11/6 Summary of reproduction toxicity studies with BAS 351 H

Study Dose levels (Batch / purity)	Endpoint	NOAEL ppm (mg/kg bw/d)	LOAEL ppm (mg/kg bw/d)	Effects at LOAEL	Reference DocID
toxicity, **oral gavage** during days 6-15 of gestation 0-22.2-66.7-200 mg/kg bw/d (batch not available/ 92.5%)	Maternal tox.	- (200, hdt)	-	No adverse effects	[redacted] (1978) Doc ID 1978/039
Rat (Wistar) Prenatal developmental toxicity, **oral gavage** during days 6-15 of gestation 0-40-100-250 mg/kg bw/d (N 187/ 97.8%)	Development	(100)	- (250)-	Increased fetal resorptions and retarded fetal development. Not teratogenic	Monograph B 5.6.2.2 [redacted] 1986 Doc ID 1986/421
	Maternal tox	- (100)	- (250)	Reduced food consumption	
Rat (SD:CRJ) Prenatal developmental toxicity, **diet during** days 0-21 of gestation 0-2,000-4,000-8,000 ppm 0-162-324-631 mg/kg bw/d (N169 / 93.9%)	Development	4,000 (324)	8,000 (631)	Reduced body weight and ossification of cervical vertebrae Not teratogenic	Monograph B 5.6.2.3 [redacted] 1982 Doc ID 1984/066
	Maternal tox.	2,000 (162)	4,000	Increased water consumption and amniotic fluid 8,000 ppm: reduced bw gain, hematuria	
Published literature: Rat (strain not given) Prenatal developmental toxicity, **single oral gavage** on day 6, 8, 11, 14 or 16 of gestation 0-25 - 90- 200 mg/kg bw **Basagran** corresponding to 0- 12- 43.2-96 mg/kg bw bentazone (batch / purity not submitted)	Development	- (200, hdt)	–	Increased resorption rate, retardation of fetal development and incomplete ossification in all treated groups. No dose-response relationship. Inconsistency of data. No assessment possible.	Monograph B 5.6.2.6 El-Mahdi MM and Lofti MM, 1988 Doc ID 1988/10538
	Maternal tox.	No data given			
Rabbit (Himalayan, ChBB:HM) Prenatal developmental toxicity, oral gavage during days 6-18 of gestation; 0-50-100-150 mg/kg bw/d (batch not available / 92.5%)	Development	- (150, hdt)	-	No adverse effects	Monograph B 5.6.2.7 [redacted] Doc ID 1984/048 [redacted] 1984 Doc ID 1984/288
	Maternal tox.	- (150, hdt)	-	No adverse effects	
Rabbit (Chinchilla) prenatal developmental toxicity, oral gavage during days 6-18 of gestation 0-75-150-375 mg/kg bw/d (N187 / 97.8%)	Development	- (150)	- (375)	Total post implantation loss in one dam. Not teratogenic	Monograph B 5.6.2.8 [redacted] 1987 Doc ID 1987/058
	Maternal tox.	- (150)	- (375)	Reduced food consumption	
Published literature: Mice (CD1) Subchronic (100 days) vs. pre natal (complete gestation) & subchronic administration via drinking water 30 µg/l	Development	30 µg/l (hdt)		No adverse effects	AIR2-Dossier II A 5.6.6/1 Garagna S. et al. 2005 Doc ID 2011/1257366
	Parental tox.	30 µg/l (hdt)		No adverse effects	

Table 5.11/6 Summary of reproduction toxicity studies with BAS 351 H

Study Dose levels (Batch / purity)	Endpoint	NOAEL ppm (mg/kg bw/d)	LOAEL ppm (mg/kg bw/d)	Effects at LOAEL	Reference DocID

hdt: highest dose tested

Changes in regard to the original monograph are indicated in bold Italian style

Several prenatal toxicity studies in rats and rabbits and two multi-generation studies in rats did not reveal a teratogenic potential or any adverse effects of bentazone on the reproductive performance.

The multigeneration studies in rats indicated developmental toxicity in form of slightly reduced pup weight gain during lactation. Based on a reassessment of the individual data it was possible to demonstrate that impaired pup weight gain was associated with litters from dams showing significant maternal toxicity based on reduced food intake and reduced body weight gain in the first period of the lactation phase. Therefore, this evaluation shows that pup weight effects were observed only at maternally toxic levels as secondary effect. Additionally, the effect levels were recalculated based on the actual food intake in the sensitive period (PND1-4), which is higher than the average food intake within the gestation. Therefore the NOAEL in the multigeneration study is newly set to 200 ppm both for developmental and maternal effects, which is calculated to be 22 mg/kg bw, and the LOAEL is set to 800 ppm (80 mg/kg bw).

In the prenatal developmental toxicity studies in rats with bentazone administration via gavage, developmental toxicity was apparent in form of delayed fetal maturation (reduced fetal weight and reduced skeletal ossification) and increased postimplantation loss (fetal resorptions) in the presence of slightly reduced maternal food consumption at a dose level of 250 mg/kg bw. A clear NOAEL was seen at 100 mg/kg bw. For bentazone, reduced food consumption has shown to be a relatively late and weak indicator of toxicity. Considering weight of evidence it is plausible that the typical target organs of bentazone blood, kidney and liver were already impaired at the high dose, which is supported by the effects achieved at this dose in the subchronic toxicity studies.
Additionally, in the *dietary* developmental study in rats with bentazone administration during the complete gestation, the NOAEL for maternal toxicity was 2000 ppm (equal to 162 mg/kg per day) on the basis of increased water consumption of the dams and increased amniotic fluid at 4000 ppm (equivalent to 324 mg/kg bw). Severe maternal effects like decreased weight gain and food consumption and signs of hemorrhagic diathesis were seen at 8000 ppm (equivalent to 631 mg/kg bw). At this dose level secondary fetal toxicity was apparent as decreased fetal weights, reduced ossification and fetal liver haemorrhages. No postimplantation losses were seen. The developmental NOAEL after dietary administration was defined at 4000 ppm (equal to 324 mg/kg bw per day). In view of these studies, fetal resorptions are considered to be peak plasma effects which are based on the rapid substance uptake and the high plasma concentrations achieved after gavage. As dietary studies did not led to fetal resorptions up to the highest dose levels it can be concluded that significant effects would not occur under realistic exposure scenarios.

Developmental toxicity in rabbits after gavage was seen in one doe with partial abortion, embryonic resorptions and no living fetuses at 375 mg/kg bw, a dose at which the dams showed additionally reduced food consumption.

Additionally, the publications reviewed within this AIR2-dossier revealed no endocrine potential of bentazone.

In summary, bentazone is considered to induce developmental toxicity in rats and rabbits only at maternal toxic doses. The data provided no indication of increased susceptibility in rats or rabbits from in utero and postnatal exposure to bentazone. Developmental toxicity appeared at maternal toxic doses. Bentazone is not teratogenic.

G. NEUROTOXICITY

Bentazone has been investigated for its neurotoxic properties in Wistar rats in a subchronic study over 91 days and did not reveal any clinical (general clinical observation, FOB and motor activity) or neurohistopathological indication of neurotoxicity.
An acute neurotoxicity study was announced for submission but was postponed as the US registration process is at a later date. The missing of this study is in view of the subchronic study not regarded as data gap.

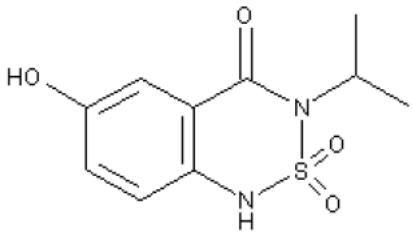
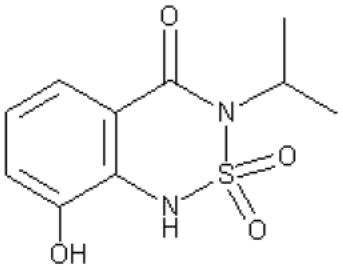
Table 5.11/7 Summary of 90-day oral rat neurotoxicity study with Bentazone

Test system Dose levels (ppm)	[batch /purity]	NOAEL mg/kg bw/d (ppm)	LOAEL mg/kg bw/d (ppm)	Effects at LOAEL	Reference (BASF DocID)
Wistar (CrlGlxBrlHan) rats, 0–300-1,000-3,500	(batch N 187, purity 96.9%)	M 258 F 306 (3,500)	No adverse effects	None	AIR2-Dossier IIA 5.7.4/1 [redacted] 2004 Doc ID 2004/1013171

H. STUDIES ON METABOLITES

Plant metabolites
Investigations on the major plant metabolites 6-Hydroxy- and 8-Hydroxybentazone, including an up-to-date AMES on 8-OH-Bentazone, revealed that the plant metabolites are less toxic than the parent substance bentazone and furthermore, do not have any mutagenic or teratogenic potential.

Figure 5.11/2 Chemical structures of major plant metabolites 6-OH and 8-OH-Bentazone

6-OH-Bentazone (M351H001, Reg.No. 108745)	8-OH-Bentazone (M351H002, Reg.No. 108746)
	

Photolysis metabolites (see Figure 5.11/2)

The photolysis metabolites of Bentazone, Peak B, Peak C and BAS 351H025, have been demonstrated to be formed in the upper surface layer and are therefore regarded to be potential candidates for human exposure. Definite data requirements regarding toxicological testing of surface-water metabolites does not exist.

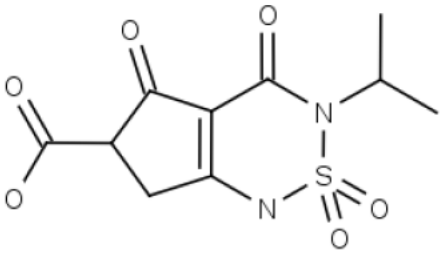
The QSAR analysis for Peak B revealed an in vitro genotoxic alert, which is known to be not relevant in vivo and therefore has little or no significance in risk assessment. Peak C had no alert and M351H025 revealed an alert for skin sensitization, which became redundant due to negligible environmental concentration values.

Soil- and surface water-metabolite N-Methylbentazone (see Figure 5.11/3)

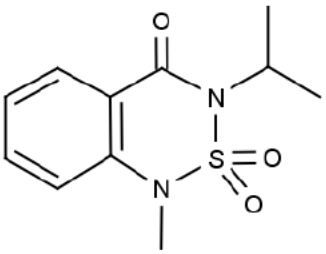
The soil- and surface water-metabolite N-Methylbentazone has been identified as a relevant metabolite based on its biological activity in the sequential assessment performed in accordance to the "Guidance document on the assessment of the relevance of metabolites in groundwater of substances regulated under council directive 91/414/EEC" (Sanco/221/2000). As relevant metabolite it has to stay below a concentration of 0.1 µg/L. No additional screening for genotoxicity was therefore relevant. The QSAR analysis using DEREK revealed no genotoxic alert for this structure.

Figure 5.11/3 Chemical structures of the photolysis metabolites Peak B, Peak C and M351H0025 and the soil and surface-water metabolite N-Methylbentazone

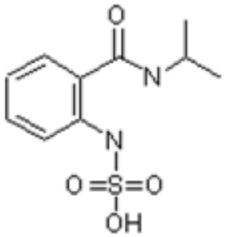
Peak B (M351H023)



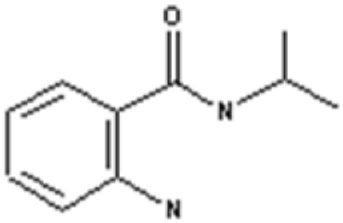
N-Methylbentazone



Peak C (M351H024, Reg.No. 268168)



M351H025 (Reg.No.36848)



I. Other/special studies

A general pharmacology study and a study on EEG effects evaluated in the monograph revealed no pathological changes by bentazone-treatment.
A mechanistic plasmakinetic study under use of Probenecid revealed that Bentazone is excreted in rats via the saturable renal Organic Anion Transporter and in a further step, that saturation of excretion starts between dose levels of 84.7 and 165.9 mg/kg bw (calculated as bentazone-sodium salt).
Furthermore based on public peer-reviewed literature, bentazone is not expected to react with nuclear cell receptors (Aryl-Hydrocarbon Receptor, the Peroxisome proliferator-activated receptors α or γ, or the human and mouse pregnane X receptor) to induce enzyme induction. Additionally, Bentazone is reported to show no effect on metabolic activity of phagocytes but seem to induce a reduction in leukocyte migration ability in vitro.

An immunotoxicity study was announced for submission but was postponed as the US registration process is at a later date. The missing of this study is not regarded as data gap as neither the signs of toxicity nor the target organs affected by bentazone did indicate immunotoxic properties.

J. HUMAN DATA

Several case reports of suicide attempts with bentazone are known, some of which resulted in deaths. The lowest reported fatal dose was 20 g; however, this information is related to a case, which occurred in China, and could not be verified. The published cases of clinical reports of human intoxication confirm the toxicological profile of bentazone found in animals, with the blood, kidney and the liver as target organ. The clinical signs after ingestion of Bentazone in humans regarding respiratory problems, vomiting, diarrhoea, muscle rigidity and abdominal pain are in line with the findings in acute toxicity studies. The changed blood parameters for creatinine, BUN and blood coagulation were also observed in animal studies. Lethality occurred between 2 hours and 5 days after ingestion at dose levels comparable to those found to be lethal in animals. The published clinical reports do not indicate that humans are more sensitive to Bentazone than animals.
No human poisoning incidents or detrimental health effects have been observed among employees having contact with the active ingredient. Some cases of irritation of the eyes and the skin have been registered in the BASF-internal clinical incident log in persons exposed to bentazone.
No other adverse health effects due to bentazone have been documented in the BASF-internal medical files.

Neither data on exposure of the general public nor epidemiologic studies are available for BASF SE, nor is BASF SE aware on any epidemiologic studies performed by third parties with bentazone.

5.11.1 Acceptable Daily Intake (ADI)

In the course of the EU registration of Bentazone the Acceptable Daily Intake (ADI) was fixed to be used for the long-term risk assessment for consumers. In the 'Review report for the active substance Bentazone' (30 November 2000; 7585/VI/97-final) finalised in the Standing Committee on Plant Health at its meeting on 13 July 2000 the following ADI was set:

ADI = 0.1 mg/kg bw/day

This value is based on the NOAEL of 10 mg/kg observed in the 24-month rat study (BASF DocID 1985/433) and a Safety Factor of 100.

This value is still valid based on the following argumentation: The data base for the above reference values did not change since the submission of the Bentazone EU Dossier in 1995 and the evaluation in 2000. The chronic toxicity studies including the 1 year dog study and the reproduction toxicity studies are considered the most relevant. The only possibly relevant change is the re-evaluation of the 2-generation study in 2011 (see II A 5.6.1/1). However, the NOAEL in this study was changed from 14 mg/kg bw/d to 22 mg/kg bw/d which is not relevant under consideration of the lowest NOAEL found in the Carcinogenicity study in rats.

The relevant NOAELs for ADI calculation and the respective LOAELS are listed in Table 5.11/8.

Table 5.11/8 Summary of combined NOAELs and LOAELs from studies relevant for establishing the ADI

Study	NOAEL		LOAEL	
	mg/kg bw/d	ppm	mg/kg bw/d	ppm
12-month dog - oral	13.1	(400 ppm)	52.3	(1,600 ppm)
24-month chronic toxicity rat - oral	17.0	(350 ppm)	76.0	(1,600 ppm)
24-month carcinogenicity rat - oral	**10.0**	**(200 ppm)**	**40.0**	**(800 ppm)**
24-month carcinogenicity mouse - oral	12.0	(100 ppm)	47.5	(400 ppm)
2-generation study rat - oral				
- monograph	~~14.0~~	(200 ppm)	~~56.0~~	(800 ppm)
- Re-evaluation (see AIR2 II A 5.6.1/1)	22.0	(200 ppm)	80.0	(800 ppm)

The lowest NOAEL from studies relevant for calculation of the ADI is still found in the long-term oral feeding study in rats with a value of 10 mg/kg bw/d. In the absence of mutagenicity, oncogenicity, teratogenicity or developmental toxicity the use of a standard safety factor of 100 is warranted, resulting in the above mentioned ADI.

5.11.2 Acute Reference Dose (ARfD)

In the course of the EU registration of Bentazone the Acute Reference Dose (ARfD) was fixed. In the 'Review report for the active substance Bentazone' (30 November 2000; 7585/VI/97-final) finalised in the Standing Committee on Plant Health at its meeting on 13 July 2000 the following ARfD was set:

ARfD = 0.25 mg/kg

This value is based on the NOAEL of 25.3 mg/kg observed in the 90-day rat study (BASF DocID 1987/0173) and a Safety Factor of 100.

The data base for the above reference value did not change since the submission of the Bentazone EU Dossier in 1995 and the evaluation in 2000. For the determination of the ARfD, short-term, developmental and neurotoxicity studies are considered to be most important. Possibly relevant changes are the subchronic neurotoxicity study (summary provided in this AIR2-dossier II A 5.7.4/1 and 2) and the new comparative subchronic study (summary provided in this AIR2-dossier II A 5.3.2/1 and 2). However, the NOAEL for neurotoxicity was 258 mg/kg and the NOAEL for Bentazone-sodium in the subchronic toxicity study was 91 mg/kg bw; thereby both studies are not relevant in view of the NOAEL in the original 90-day rat study used for ARfD setting.

It is worth to note that at the 2004 JMPR meeting in Rome, Italy (20–29 September 2004) it was concluded that the establishment of an ARfD was unnecessary for Bentazone[i]. This suggestion was taken up by the notifier within the course of the JMPR Evaluation of Bentazone provided in 2011 with the following argumentation:

An evaluation of the toxicological database for bentazone shows that bentazone is only moderately toxic after acute oral exposure with a lethal dose above 1,000 mg/kg bw in rats (LD_{50} was 1470 mg/kg bw in the most relevant study).
Predominant target after short-term to subchronic oral exposure of rats, mice and dogs was the blood coagulation system (prolonged blood coagulation time, diminished hematocrit, reduced haemoglobin, subsequent haemorrhages at higher dose levels). Although the mode of action of bentazone for the effects on the blood coagulation system is not known, it is highly unlikely that a single low dose of bentazone results in adverse effects. Due to the rapid excretion of bentazone no prolonged effect of a single low dose on the coagulation system is likely. Therefore, the elongation of prothrombin and partial prothrombin time observed in short-term studies are not suitable parameters for the determination of an ARfD. Therefore, different parameters have to be considered.
Other findings in short-term to subchronic studies in rats, mice and dogs included mortality at high dose levels (rats at ~ 250 mg/kg bw/day, mice at ~ 900 mg/kg bw/day and dogs at ~114 mg/kg bw/day), reduced body weight gain and organ weight changes. However, these parameters are not considered to be suitable for the determination of an ARfD since mortality was generally observed towards the end of the treatment period and therefore not an acute effect while effects on body and organ weights are unlikely to result from a single application.

Further toxicological effects in the sub-chronic rat study were changes in clinical chemistry parameters (increased plasma cholesterol and albumin levels) at the LOEL (1200 ppm) and increased urinary output at 3600 ppm. These parameters are indicative for some effects on liver and kidney; however, examinations revealed no histopathological changes at any dose level.
In the 90-day dog study, histopathological lesions were found (severe congestive symptoms, necrotic congestion, marked fatty degeneration of the liver, fatty degeneration in the ventricular myocardium and the albuminous swelling of the renal tubules) at 3000 ppm resulting in substantial toxicity (as indicated by the death of 3 out of 6 dogs) and thus are not the consequence of a single administration of bentazone. In the 1-year study in dogs, clinical signs (emaciation, dehydration, hyperaemia, alopecia and (occasionally bloody) diarrhoea were seen at the highest dietary concentration. The NOAEL for this study was 400 ppm (equal to 13.1 mg/kg bw per day) on the basis of clinical signs, body weight loss and anaemia at the highest dietary concentration. It is considered not appropriate to set an ARfD on the clinical signs, reduced body weight or haematological changes occurring in dogs, since significant clinical effects were not seen early in these two studies.

Two prenatal developmental studies with administration by gavage and one dietary developmental study were performed in rats. In the earlier study of rats treated by gavage, neither maternal nor fetal toxicity was seen at any dose; the NOAEL for both maternal and fetal toxicity was thus 200 mg/kg bw per day, the highest dose tested. In the later gavage study, in which higher doses were administered, the NOAEL was 100 mg/kg bw per day on the basis of maternal toxicity (decreased food consumption) and fetal toxicity (postimplantation loss, reduced fetal weight and incompletely ossified fetal skeletons). In the dietary developmental study in rats with bentazone administration during the complete gestation, the NOEL for maternal toxicity was 2,000 ppm (equal to 162 mg/kg per day) on the basis of increased water consumption and increased amniotic fluid at 4,000 ppm (equivalent to 324 mg/kg bw). Severe maternal effects like decreased weight gain and food consumption and signs of hemorrhagic diathesis were seen at 8000 ppm (equivalent to 631 mg/kg bw). At this dose level secondary fetal toxicity was apparent as decreased fetal weights, reduced ossification and fetal liver haemorrhages. No postimplantation losses were seen. The NOAEL for fetal toxicity was defined at 4000 ppm (equal to 324 mg/kg bw per day). Bentazone was not teratogenic in any of the studies of developmental toxicity in rats. These effects are not considered relevant for ARfD setting since the dietary exposure is considered more relevant for human exposure than the gavage administration. Postimplantation losses were not induced by dietary administration up to doses of 631 mg/kg bw/day. The effects seen like decreased fetal weights and reduced ossification are considered to be secondary to maternal toxicity and are therefore no acute effects. Furthermore, the expected human exposure due to bentazone residues is lower by several orders of magnitude.

The two developmental toxicity studies in rabbits, both gavage studies, revealed in combination a NOAEL for maternal toxicity at 150 mg/kg bw/day on the basis of reduced maternal food consumption at 375 mg/kg bw per day. Postimplantation losses were increased at 375 mg/kg bw per day based on one total postimplantation loss in one dam. Bentazone was not teratogenic in either study of developmental toxicity in rabbits. Based on the similarity to the rat effects and under consideration of the lesser sensitivity of rabbits compared to rats, the results are not considered relevant for derivation of an ARfD.

Several case reports of fatal self-poisoning in humans were characterized by vomiting, diarrhea, drowsiness and death from cardiac arrest. The lowest reported dose associated with a death was 20 g; however, this information is related to a case, which occurred in China, and could not be verified.

As a conclusion, based on the acute, short-term and developmental toxic effects of bentazone, the derivation of an ARfD is not considered to be required.

5.11.3 Acceptable Operator Exposure level (AOEL)

In the course of the EU registration of Bentazone the Acceptabel Operator Exposure level (AOEL) was fixed. In the 'Review report for the active substance Bentazone' (30 November 2000; 7585/VI/97-final) finalised in the Standing Committee on Plant Health at its meeting on 13 July 2000 the following AOEL was set:

AOEL = 0.13 mg/kg

This value is based on the NOAEL of 13.1 mg/kg observed in the 1-year dog study (BASF DocID 1989/0049 and DocID 1989/0153) and a Safety Factor of 100.

The data base for the above reference value did not change since the submission of the Bentazone EU Dossier in 1995 and the evaluation in 2000. For the determination of the AOEL, short-term, developmental, reproduction and neurotoxicity studies are considered to be most important. Possibly relevant changes are the subchronic neurotoxicity study (summary provided in this AIR2-dossier II A 5.7.4/1 and 2) and the new comparative subchronic study (summary provided in this AIR2-dossier II A 5.3.2/1 and 2). However, the NOAEL for neurotoxicity was 258 mg/kg and the NOAEL for Bentazone-sodium in the subchronic toxicity study was 91 mg/kg bw; thereby both studies are not relevant in view of the NOAEL in the original 1-year dog study used for ARfD setting. Furthermore, the literature research did not indicate that humans are more sensitive.

Glossary

Terms and abbreviations

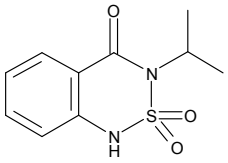
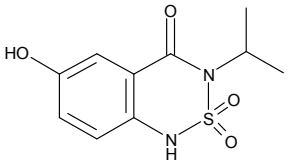
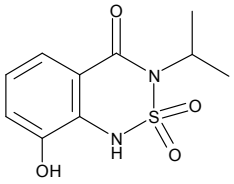
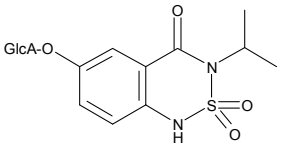
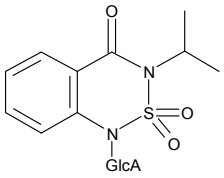
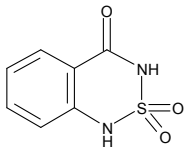
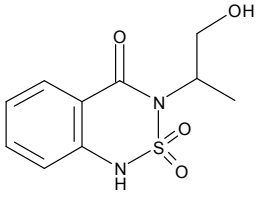
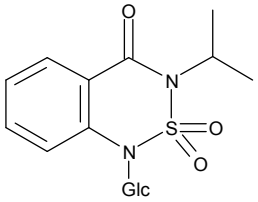
AUC	Area under the curve
bw	body weight
CHO	Chinese hamster ovary
CMC	carboxymethylcellulose
DEN	N-nitrosodiethylamine; diethylnitrosamine
F or ♀	Female
HPRT	Hypoxanthine-guanine phosphoribosyl transferase
M or ♂	Male
MN	micronuclei
NCEs	normochromatic erythrocytes
p.c.	post coitum
PCEs	polychromatic erythrocytes
p.i.	post insemination
PND	post natal day
p.p.	post partum
SL	Soluble concentrate
UDS	Unscheduled DNA synthesis

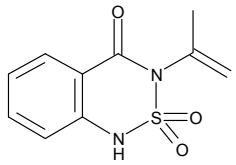
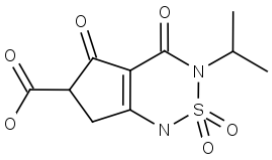
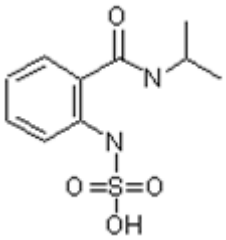
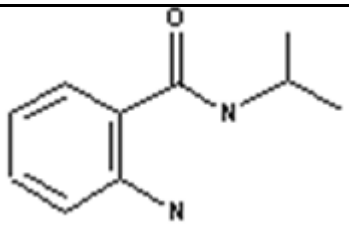
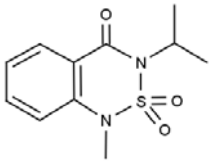
List of concordances for names and designations of compounds

Active substances

Other names / codes used	Comments
Bentazone	Alternative notations
Bentazon	
BAS 351 H	BASF internal code number for the active substance as free acid
BAS 351 H-Na	BASF internal code number for the active substance as sodium salt
Reg. No. 51929	alternative BASF internal code number for the active substance as free acid
Reg. No. 88691	alternative BASF internal code number for the active substance as sodium salt
Bentazone Na-salt	Alternative notations for the sodium salt of Bentazone
Bentazone-Na	
Bentazone sodium salt	
BAS 351 32 H	BASF internal code number for a formulation
Basagran	Trade Name for BAS 351 32 H

Metabolites

Chemical name	Reg.No.	Molecular structure	Metabolite code
Bentazone 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide	51929		BAS 351 H M351H000
6-OH-Bentazone	108745		M351H001
8-OH-Bentazone	108746		M351H002
not defined	not defined		M351H003
not defined	281174		M351H004
not defined	76183		M351H005
not defined	not defined		M351H006
not defined	not defined		M351H007

not defined	not defined		M351H008
Peak B	5819746		M351H023
Peak C	268168		M351H024
not defined	36848		M351H025
N-Methylbentazone	79520		M351H009

[i] FAO, WHO, Pesticide residues in food - 2004, Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues, FAO Plant Production And Protection Paper 178, Page 39, http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/DOWNLOAD/2004_rep/report2004jmpr.pdf

BASF
The Chemical Company

DOSSIER FOR THE EVALUATION OF THE ACTIVE SUBSTANCE

Bentazone (BAS 351 H)

Data requirement according to Regulation (EU) No. 544/2011 and (EU) No. 545/2011 (formerly Annex II and III of Directive 91/414/EEC) using OECD Guidance for Industry Data Submissions on Plant Protection Products and their Active Substance (Dossier Guidance)

Document M-II

Summary and evaluation (Tier II)

Section 4

Residues and plant metabolism

BASF DocID 2012/1007334

compiled by

[redacted]

BASF SE

[redacted]

Date: 24 February 2012

6 Metabolism and Residues Data

List of Contents

List of Contents - continued

In the following chapters, sometimes a reference to the Full Report (1998) is given. The full citation to this document reads "Full Report of the Peer Review Meetings organised by the ECCO teams for Bentazone, 1998".

According to Article 1(c) of Regulation (EU) No. 1141/2010 the supplementary dossier includes data and risk assessments which were not part of the original dossier and which are necessary to reflect changes. Reasoning for each test or study as required by Art 1(d)/(e) of the Regulation is given in the reference list of the dossier.

For Bentazone a literature search has been performed. The search report including a description of the selection and assessment process is provided in BASF DocID 2012/1007281 (DocLIIA).
The results of the selection process and assessments for Residues and plant metabolism are outlined in BASF DocID 2012/1007286.

6.1 Stability of residues

For the active substance bentazone, data on the stability of residues were reviewed during the Annex I inclusion process. The stability of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone was investigated in samples with occurring residues through reanalysis. However, according to the Full Report (Full Report of the Peer Review Meetings by ECCO for Bentazone (1998)), the stability of residues requires assessment if samples are stored prior to analysis. Therefore, a new storage stability study was conducted and is submitted in this dossier. In this study, bentazone and the glucoside derivatives of 6-OH- and 8-OH-bentazone were spiked in different plant matrices covering the four relevant categories for bentazone uses, such as maize green plant (high water), grain (starch) and straw, pea seed (protein), flax seed (oil) and potato tuber (starch), showing stability over a period of two years. Since no residues of bentazone and and the conjugated metabolites of 6-OH- and 8-OH-bentazone above the LOQ are expected in animal matrices, a stability study of residues in products of animal origin is not considered necessary.

6.1.1 Stability of residues during storage of samples

Report:	II A 6.1.1/1 Sasturain J. et al. 2002(b) Investigation of the stability of residues of Bentazone and its metabolites 6-OH-Bentazone and 8-OH-Bentazone as glucoside derivatives in plant matrices under normal storage conditions BASF DocID 2002/1008779
Guidelines:	EPA 171-4(e); IVA Guideline Residue Chemistry Part II Storage Stability 1992
GLP:	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

The deep-freeze stability of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone as glucoside derivatives has been investigated in different plant matrices such as maize (green plant, grain and straw), pea (seed), flax (seed) and potato (tuber) over a period of two years. The matrix samples were spiked with the test substances at a concentration of 0.5 mg/kg and analysed in duplicate after different storage intervals by GC-MS. Analytical results demonstrated that bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone as glucoside derivatives, were stable in the different plant matrices over the test period of two years.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: Bentazone;
BH 351-6-OH / 6-OH-bentazone;
BH 351-8-OH / 8-OH-bentazone
Lot/Batch #:
Purity: Bentazone: 99.6%
CAS#: Bentazone: 25057-89-0
Development code: Bentazone: BAS 351 H
Spiking levels: 0.5 mg/kg

2. Test Commodity:

Crop: Maize, potato, flax, pea
Type: No data
Variety: No data
Botanical name: Maize: *Zea mays*
Potato: *Solanum tuberosum*
Flax: *Linum usitatissimum*
Pea: *Pisum sativum*
Crop parts(s) or processed commodity: Maize: green plant, grain and straw
Potato: tuber
Flax: seed
Pea: seed
Sample size: Not reported

B. STUDY DESIGN

1. Test procedure

The deep freeze storage stability of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone, determined as glucoside derivatives, were investigated over a period of two years in different plant matrices such as maize (green plant, grain and straw), pea (seed), flax (seed) and potato (tuber).
The glucoside derivatives of 6-OH-bentazone and 8-OH-bentazone were produced in wheat cell suspension cultures and rape cell suspension cultures, respectively. Previous attempts to produce the glucosides chemically were not successful.
Untreated matrix samples were fortified with 0.5 mg/kg of the test substances and stored at -20°C in the dark. The storage conditions correspond to the usual storage conditions for field samples. Samples were analysed after 0, 27, 97, 180, 355, 539 and 713 days.

2. Description of analytical procedures

In principle, bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone are extracted from plant matrices with aqueous methanol. After purification of a 4% aliquot by an iso-octane liquid-liquid partition, the two relevant metabolites which are present as glucosides are hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic plant constituents, a reversed phase C_{18}-column clean-up is performed. The analytes are then methylated with diazomethane and their derivatives are purified using a silica gel-column. The final determination of the residues of bentazone and its hydroxy metabolites is performed by GC-MS. The limit of quantitation (LOQ) of the method for bentazone, 6-OH-bentazone and 8-OH-bentazone is 0.02 mg/kg each.

II. RESULTS AND DISCUSSION

Procedural recoveries for bentazone averaged at about 90% for maize plant, at 94% for grain, at 97% for straw, at 87% for potato tuber, at 98% for flax seed and at 88% for pea. The mean recoveries for 6-OH-bentazone were about 77% in maize plant, 81% in grain, 83% in straw, 76% in potato tuber, 79% in flax seed and 74% in pea. Recoveries for 8-OH-bentazone averaged at about 72% in maize plant, at 73% in grain, at 80% in straw, at 72% in potato tuber, at 76% in flax seed and at 75% in pea.

Table 6.1/1 Average procedural recoveries for bentazone, 6-OH-bentazone and 8-OH-bentazone in different plant matrices

Plant Matrix	Average Procedural Recovery [%]		
	Bentazone	6-OH-bentazone	8-OH-bentazone
Maize plant	90	77	72
Maize grain	94	81	73
Maize straw	97	83	80
Potato tuber	87	76	72
Flax seed	98	79	76
Pea seed	88	74	75

Recoveries in stored samples for bentazone showed stability (>70%) for 713 and 722 days in maize plant, grain, straw, in flax seed and in pea. Only in potato the recovery dropped to 66% but this was correlating with a low procedural recovery. The recoveries in stored samples for 6-OH-bentazone and 8-OH-bentazone were well above 70% in maize plant, grain, straw, potato tuber, flax seed and in pea after 713-722 days of storage.

Table 6.1/2 Recoveries for bentazone, 6-OH-bentazone and 8-OH-bentazone in stored and freshly spiked plant matrix samples

Day	Maize, Green Plant		Maize, Grain		Maize, Straw		Potato, Tuber		Flax, Seed		Pea, Seed	
	A: in stored samples, % of nominal					B: procedural in freshly spiked sample						
	A	B	**A**	B	**A**	B	**A**	B	**A**	B	**A**	B
Bentazone												
0	**100**	107	**110**	91	**122**	115	**93**	93	**106**	93	**89**	89
27	**-***	76	**-***	109	**95**	83	**88**	84	**87**	80	**81**	73
97	**84**	84	**98**	92	**98**	95	**93**	81	**95**	105	**82**	88
180	**85**	84	**93**	91	**116**	92	**92**	91	**99**	96	**103**	91
355/356	**86**	89	**91**	83	**105**	90	**84**	82	**97**	99	**97**	89
538/539	**101**	105	**110**	99	**127**	105	**78**	98	**104**	96	**116**	86
547	**-**	-	**-**	-	**-**	-	**-**	-	**132**	124	**103**	98
713	**96**	86	**109**	97	**111**	102	**60**	79	**105**	89	**95**	90
722	**-**	-	**-**	-	**-**	-	**66**	87	**-**	-	**101**	92
6-OH-bentazone												
0	**90**	85	**109**	84	**124**	97	**85**	61	**101**	76	**95**	68
27	**-***	82	**-***	95	**107**	86	**81**	77	**97**	82	**97**	85
97	**75**	69	**104**	84	**102**	77	**91**	77	**96**	102	**81**	76
180	**86**	71	**92**	79	**132**	81	**87**	75	**93**	79	**108**	75
355/356	**85**	79	**88**	71	**96**	73	**71**	69	**95**	72	**94**	68
538/539	**98**	79	**113**	86	**123**	91	**52**	101	**118**	70	**145**	74
547	**-**	-	**-**	-	**-**	-	**-**	-	**125**	75	**119**	64
713	**83**	72	**96**	70	**100**	78	**88**	78	**118**	79	**111**	90
722	**-**	-	**-**	-	**-**	-	**92**	71	**-**	-	**116**	70
8-OH-bentazone												
0	**86**	74	**88**	62	**42**	93	**46**	45	**79**	64	**91**	54
27	**-***	69	**-***	75	**84**	70	**71**	70	**73**	69	**86**	59
97	**79**	60	**97**	80	**76**	65	**97**	62	**90**	83	**79**	67
180	**85**	68	**82**	72	**56**	77	**68**	70	**75**	72	**114**	77
355/356	**39**	73	**66**	69	**53**	78	**40**	67	**56**	72	**94**	76
538/539	**97**	79	**121**	72	**72**	83	**56**	113	**99**	76	**160**	82
547	**-**	-	**-**	-	**-**	-	**-**	-	**130**	91	**123**	94
713	**116**	79	**120**	84	**81**	92	**95**	73	**126**	80	**114**	88
722	**-**	-	**-**	-	**-**	-	**113**	79	**-**	-	**129**	81

* residues are outlier, results not used for calculation

III. CONCLUSION

Residues of bentazone and glucoside conjugates of 6-OH-bentazone and 8-OH-bentazone were stable in all plant matrices tested for up to two years. Acceptable degradation of bentazone was observed in potato tubers.

6.1.2 Stability of residues in samples extracts

Report: II A 6.1.2/1
Klaas P., Ziske J. 2009(b)
Study on the residue behaviour of Bentazone in maize after treatment with BAS 351 40 H under field conditions in Germany, Northern France, Southern France and Spain, 2008
BASF DocID 2009/1024805

Guidelines: EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8); EEC 1607/VI/97 rev. 2 10.06.1999; EEC 7029/VI/95 rev. 5; EEC 7525/VI/95 rev. 7

GLP: Yes
(laboratory certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

A residue study was conducted to determine the magnitude of residues of BAS 351 H (bentazone) in maize. A full summary of this study regarding residues after bentazone application is given in chapter 6.3. In the context of the study, stability investigations of the analytes in working solutions were performed; the results are presented below.

According to Method No. L0044/02, plant material is extracted using methanol. For the preparation of the final volume, a mixture of methanol/water (1/2) is used.
Using a maize grain control sample, tests were performed to investigate the stability of the analytes in the extract solution and in the final volume. For that purpose, the sample was fortified with 0.01 mg/kg of the analytes and the respective solutions were stored in the refrigerator and re-analysed after certain storage intervals. The results are expressed as percent recovery and summarized in the table below.

Table 6.1/3 Stability tests

Test Item	AP No.	Sample No.	Storage Time (days)	Recovery (%)		
				Bentazone	6-OH-bentazone	8-OH-bentazone
Fortified grain sample	L008	ForL0021	0	79.8	114.2	93.7
		ForL0022		78.8	110.6	85.9
		ForL0023		84.8	118.4	85.3
Extract solution	L010	ForL0029	4	78.5	94.0	107.3
		ForL0030		72.1	107.0	91.5
		ForL0031		78.9	104.2	99.1
Final volume	L010	ForL0032	5	94.4	126.6	93.8
		ForL0033		87.6	123.2	90.0
		ForL0034		99.8	120.2	86.0
	L012	ForL0041	7	90.4	109.7	98.9
		ForL0042		87.6	114.1	78.1
		ForL0043		92.8	115.5	89.3
	L014	ForL0047	12	90.6	91.2	94.6
		ForL0048		81.6	93.0	75.8
		ForL0049		86.4	93.8	75.8

It was found that storage in the final volume is possible for a time interval of at least 12 days under refrigerated conditions. In the extract solution the analytes are stable for at least 4 days.

Report:	II A 6.1.2/2 Rabe U., Kloeppner U. 2011(a) Metabolism of BAS 351 H in wheat BASF DocID 2010/1062115
Guidelines:	EPA 860.1300: EPA Residue Chemistry Test Guidelines; EPA 860.1300: Nature of the Residue in Plants Livestock; EPA 860.1000: EPA Residue Chemistry Test Guidelines; PMRA Residue Chemistry Guidelines Section 97.2 Nature of the Residue - Plants - Livestock (Canada); BBA IV 3-2; EEC 7028/VI/95 rev. 3 Appendix A (EU): Metabolism and distribution in plants (draft of 22 July 1997)
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

A wheat metabolism study was performed with ^{14}C-bentazone (^{14}C-BAS 351 H). The objective of this study was to generate data on the degradation and fate of ^{14}C-BAS 351 H in wheat. In the context of the study, stability investigations were performed; the results are summarised below.

All samples were stored in a freezer at -18°C until used. A comparison of the extractability and of the HPLC chromatograms of the metabolite patterns obtained at the beginning and at the end of the investigation period showed that there was no relevant change in the nature of radioactive residues during sample storage over a period of approximately 17 months in the extract and about 21 months in matrix.

6.2 Metabolism, distribution and expression of residues

The data evaluated during the Annex I inclusion of the active substance is sufficient to describe the general metabolic behaviour of bentazone. In the Full Report (1998), no further studies were considered necessary. Nevertheless, new analytical techniques and implementation of new guidelines for metabolism studies made higher ID rates and use of state-of-the-art analytics for identification of metabolites desirable. Therefore, it was agreed with former RMS Germany to conduct a wheat metabolism study to verify the results of previous plant metabolism studies. This new plant metabolism study is summarised under 6.2.1 below.

An overview of all metabolites including structures, names, codes and synonyms can be found in Tab 6.11/1 (M-II, 6.11).

The following end points are presented in the Full Report (1998):

Metabolism in plants (Annex IIA, point 6.1 and 6.7, Annex IIIA, point 8.1 and 8.6)

Plant groups covered	Soya, rice, maize, green beans, potatoes, cell cultures
Rotational crops	Chard, turnip, sorghum, radish, wheat
Plant residue definition for monitoring	Sum of bentazone and the conjugates of 6-OH- and 8-OH-bentazone expressed as bentazone equivalents
Plant residue definition for risk assessment	Sum of bentazone and the conjugates of 6-OH- and 8-OH-bentazone expressed as bentazone equivalents
Conversion factor (monitoring to risk assessment)	Not applicable

Metabolism in livestock (Annex IIA, point 6.2 and 6.7, Annex IIIA, point 8.1 and 8.6)

Animals covered	Lactating goats, laying hens
Animal residue definition for monitoring	Sum of bentazone and the conjugates of 6-OH- and 8-OH-bentazone expressed as bentazone equivalents
Animal residue definition for risk assessment	Sum of bentazone and the conjugates of 6-OH- and 8-OH-bentazone expressed as bentazone equivalents
Conversion factor (monitoring to risk assessment)	Not applicable
Metabolism in rat and ruminant similar (yes/no)	Yes
Fat soluble residue: (yes/no)	Comment from notifier required. (log P o/w indicates no, studies indicate yes)

6.2.1 In plants, at least three crops from three different crop categories

In the following, a summary of the bentazone metabolism in plants is given. An overview of all metabolites including structures, names, codes and synonyms can be found in Tab 6.11/1 (M-II, 6.11).

Plant metabolism studies were conducted with bentazone in potato, maize, green beans, rice and soybean, respectively, all of which were considered appropriate in the initial evaluation. The following conclusions were drawn for the metabolism of bentazone in plants:

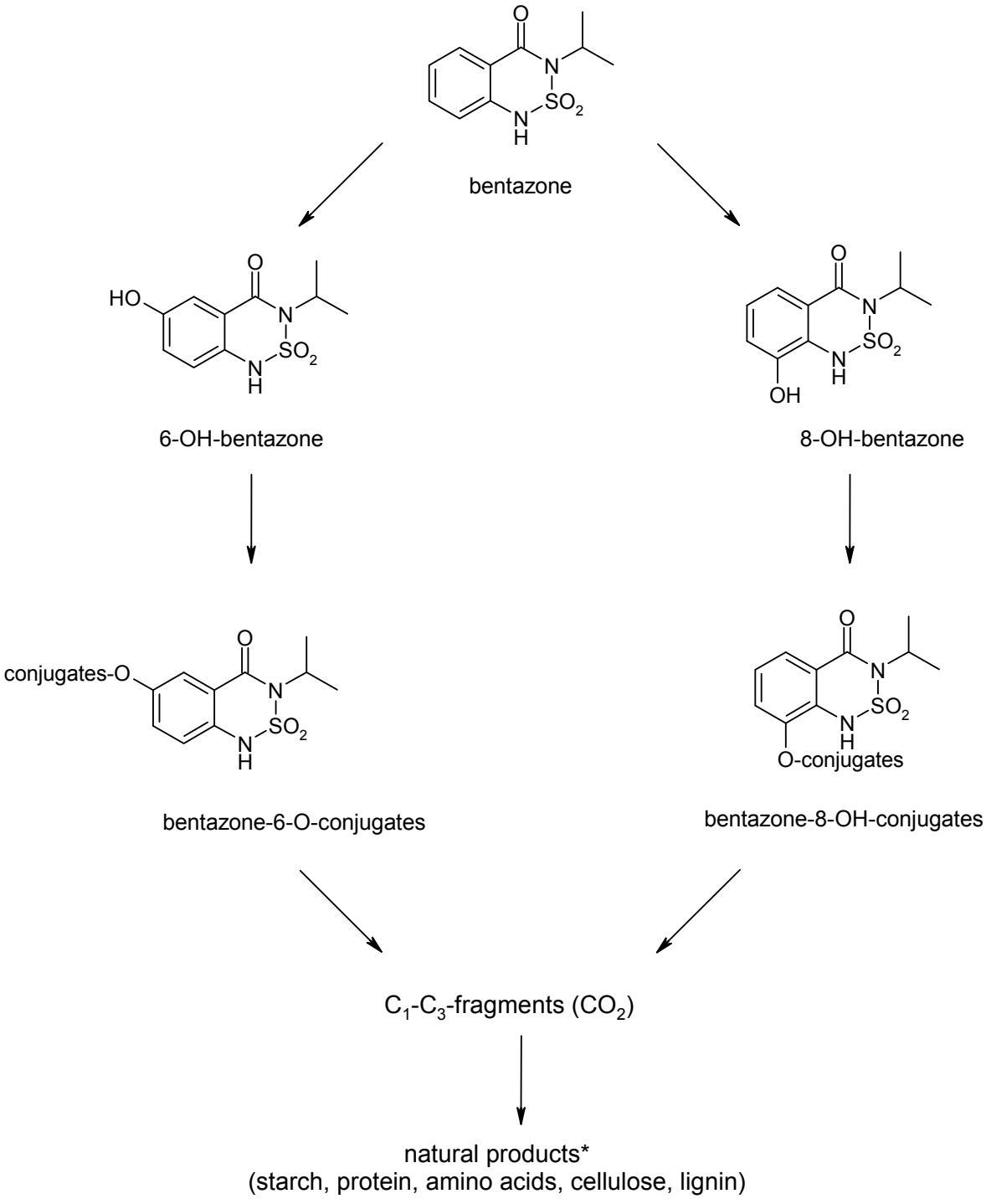
- Bentazone and the glucose conjugates of 6-OH- and 8-OH-bentazone are the main residues. No further metabolite exceeded 10% TRR. Furthermore, complete degradation of bentazone to natural products such as sugar/starch, lignin and protein was described as main metabolic route.
- The metabolism of bentazone in plants is well characterised and the sum of bentazone and the conjugates of 6-OH- and 8-OH-bentazone expressed as bentazone equivalents were defined as components of the food residue (see chapter 6.7 relative to the definition of the residue)

One additional wheat metabolism study was conducted to verify the results of previous plant metabolism studies with state of the art analytics and is summarised below.

The metabolism and distribution in plants of bentazone was investigated using ^{14}C-bentazone. In summary, the metabolic pathway of bentazone includes oxidation to 6-OH- and 8-OH-bentazone and subsequent conjugation with sugars. Further degradation leads to CO_2 and C_1-C_3-fragments, which are incorporated into natural products such as starch, protein, amino acids, cellulose and lignin. Application rates ranged from 1 kg/ha to 2.24 kg/ha for the five plant metabolism studies submitted for the initial evaluation.

In potato, bentazone and 6-OH-bentazone accounted for 4% and 12% of the total radioactive residue (TRR), respectively; 57% TRR were incorporated into starch.
In maize, no detectable residues (<0.05 mg/kg) of bentazone, 6-OH- and 8-OH-bentazone were found in grain, cobs, husks and stover, while bentazone and 6-OH-bentazone were detected in forage. A small amount of radioactivity was determined in protein, cellulose, water soluble carbohydrates and lignin.
No compounds were identified in green beans. It was anticipated that the radioactive residues found in the seed were natural products with incorporation of radioactivity.
The majority of the radioactivity in rice grain (70% TRR) was incorporated into starch, while in rice straw, the radioactivity was incorporated into unspecified polysaccharides (28% TRR) and associated with the lignin pool (24% TRR) and cellulose (1% TRR). 14% TRR were identified as bentazone, 8% TRR as 6-OH-bentazone.
In soybean, significant losses (up to 50%) of TRR going from mature to dried plants were observed. This finding was attributed to $^{14}CO_2$-respiration resulting from the conversion of ^{14}C-bentazone residues to ^{14}C-natural products *via* the carbon pool. Less than 0.1% of the applied material was found in seed. Bentazone, 6-OH and 8-OH-bentazone as conjugated glucosides accounted for 80% and 110% of the forage and hay extractable radioactive residues (ERR), respectively. The residual radioactive residues (RRR) of forage and hay were associated with water soluble polysaccharides and hemicellulose at about 60 and 30% each. The TRR associated with the seed protein and carbohydrates was 57 and 16% TRR, respectively.

Figure 6.2/1 Proposed metabolic pathway of bentazone in plants (potato, soybean, rice, maize, green bean)



* do not represent "bound residues", as by IUPAC definition

Report:	II A 6.2.1/1 Rabe U., Kloeppner U. 2011(a) Metabolism of BAS 351 H in wheat BASF DocID 2010/1062115
Guidelines:	EPA 860.1300: EPA Residue Chemistry Test Guidelines; EPA 860.1300: Nature of the Residue in Plants Livestock; EPA 860.1000: EPA Residue Chemistry Test Guidelines; PMRA Residue Chemistry Guidelines Section 97.2 Nature of the Residue - Plants - Livestock (Canada); BBA IV 3-2; EEC 7028/VI/95 rev. 3 Appendix A (EU): Metabolism and distribution in plants (draft of 22 July 1997)
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

A wheat metabolism study was performed with ^{14}C-bentazone (^{14}C-BAS 351 H). The active substance was applied once at an application rate of 1 kg a.s./ha, representing the critical GAP. Samples of wheat forage and hay were collected at BBCH 39 (20 days after application) and samples of grain, chaff and straw were sampled at BBCH 89 (83 days after application).

The total radioactive residues (TRR) were determined by summing the extractable radioactive residues (ERR) and the residual radioactive residues (RRR) after solvent extraction. The calculated TRR for wheat forage accounted for 4.461 mg/kg. Wheat hay showed the highest residue level of all matrices at 30.913 mg/kg, followed by wheat straw with residue levels of 17.315 mg/kg. In wheat chaff the residues amounted to 1.555 mg/kg while lowest residue levels were found in wheat grain at 1.112 mg/kg.

Extractabilities were tested by successive extraction with methanol and water. The extractability of radioactive residues was high for wheat forage, hay and straw with 88.6 %, 82.6 % and 71.5 % TRR, respectively. In contrast, low extractabilities were observed for wheat chaff and grain with portions of 20.5 % and 9.5 % TRR, respectively. All matrices except grain showed major extraction of radioactive residues with methanol while only minor portions were extracted with water. For grain equal amounts of residues were found in methanol and water extracts indicating high water solubility of the grain residues.
The residual radioactive residues (RRR) after solvent extraction of wheat forage, hay and straw represented portions of 11.4 %, 17.4 % and 28.5 % TRR, respectively. In the case of wheat chaff and grain, the RRR accounted for 79.5 % and 90.5 % TRR, respectively.

The parent compound was found to be moderately metabolised until harvest. Portions between approximately 39% and 56% of the total radioactive residues were still present as unchanged active substance BAS 351 H in forage, hay and straw. The major metabolite in quantitative terms was an *O*-monosaccharide conjugate of a 6-hydroxylated derivative of parent compound (M351H013). Further components also generated by hydroxylation of the benzothiadiazine ring and subsequent conjugation with monosaccharide or with a methyl group were detected only in minor amounts. In addition, the benzothiadiazine ring was conjugated with monosaccharide / disaccharide, in which the sugar moiety is directly attached to the free sulfonamide nitrogen. Decreasing extractability of the mature sample material indicated that transformation products were also incorporated into insoluble cell wall polymers and starch. In addition, radiolabelled carbohydrates (mainly glucose) were found, particularly in grain, which results from complete degradation of the phenyl ring into compounds suitable for the biosynthesis of carbohydrates and re-assimilation into natural compounds.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** Bentazone
   Description: ^{14}C in the Phenyl ring ("Phenyl label"; Phenyl-U-^{14}C); specific activity 317400 dpm/µg
   Lot/Batch #: 210-2201
   Purity: Radiochemical purity: 97.3%
   Chemical purity: 95.2%
   CAS#: 25057-89-0
   Development code: BAS 351 H
   Stability of test compound: The test item was stable over the test period

2. **Test Commodity:**
   Crop: Wheat
   Type: Cereals
   Variety: Thassos
   Botanical name: *Triticum aestivum*
   Crop parts(s) or processed commodity: Forage, hay, grain, chaff and straw
   Sample size: Not relevant

3. **Soil:** A silty loamy sand (Bruch West) (DIN 4220) was used (USDA loam). The soil physicochemical properties are described below (Table 6.2/1).

Table 6.2/1 Soil Physicochemical Properties

Soil Series	Soil Type	pH	TOC%	Sand%	Silt%	Clay%	Maximal Water Holding Capacity	CEC $cmol^+/kg$
Not reported	Loam*	7.5**	1.86	45.6*	38.4*	16.1*	31.7 g / 100 g dry soil	16.3

* USDA scheme ** ($CaCl_2$)
TOC total organic carbon
CEC cation exchange capacity

B. STUDY DESIGN

The study was conducted during the period of January 2009 to March 2011 by BASF Agricultural Center, Limburgerhof, Germany.

1. Test procedure

The metabolism of bentazone was investigated in wheat. The cultivation of the crop and the plant uptake part of the study took place in plastic containers located in a phytotron. The phytotrons simulated the natural climatic conditions for a typical spring wheat growing area. Wheat was sowed into plastic containers (size: 0.365 x 0.56 m = 0.2044 m^2; total test area = 2.04 m^2) filled with sandy loam soil (USDA scheme). The maintenance of the crop was performed in accordance with normal agricultural practice; fertilizers and plant protection products were applied to achieve an adequate plant growth. According to the planned GAP, the crop was treated with ^{14}C-BAS 351 H at a nominal rate of 1000 g a.s./ha. The foliar application took place at BBCH growth stage 31-32 using an automatic spray track system. Samples of wheat forage and hay were taken 20 days after application (20 DAT, BBCH 39). Harvest of wheat straw, chaff and grain was accomplished 83 DAT (BBCH 89).

2. Description of analytical procedures

Prior to extraction and determination of the total radioactive residues (TRR), subsamples of wheat chaff and grain were homogenised. Frozen subsamples of all other matrices were mixed with dry ice and homogenised. The homogenised material was transferred into polycarbon boxes and stored at –18°C or below. In order to determine the TRR values by combustion analysis, small aliquots of the homogenised subsamples were combusted to $^{14}CO_2$.

Subsamples of homogenised plant material were extracted three times with a sufficient volume of methanol. After each extraction step, the liquid phase was separated from the solid, and the remaining plant material was subjected to the next extraction step. The methanol extracts of the three steps were combined, adjusted to a defined volume and measured by liquid scintillation counting (LSC). The residue was extracted two additional times in the same way with appropriate volumes of water. The aqueous extracts were also combined, adjusted to a defined volume with water, and aliquots of the combined aqueous extract were radioassayed by LSC. Results of methanol and water extractions are referred to as extractable radioactive residues (ERR).

The residue after solvent extraction (methanol and water) of each sample was deep frozen and freeze died, and the weight of the remaining sample was determined. The samples were homogenised prior to combustion analysis of aliquots for the determination of the residual radioactive residues (RRR).

The total radioactive residues (TRR) were the result of the combustion of aliquots or the sum of ERR and RRR values.

The identification of the metabolites is based on LC-MS and LC-MS/MS analyses of fractions isolated from the methanol extract of wheat hay and on co-chromatography of the obtained extracts with reference items. Peak assignment in the other samples was achieved by comparison of the HPLC retention times and comparison of the metabolite patterns with those of the extracts investigated by LC-MS and LC-MS/MS and co-chromatography. Quantitation of all degradation products was based on the radio-HPLC results of the combined methanol and aqueous extracts as well as on the radio-HPLC analyses obtained from the supernatants of the aqueous ammonia treatments and enzyme treatments of the RRR.

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

Following foliar application of bentazone to wheat, the total ^{14}C-residue (TRR) was measured in wheat forage, hay, straw, chaff and grain (see Table 6.2/2).

Table 6.2/2 Total radioactive residues in wheat samples after foliar treatment with ^{14}C-BAS 351 H

Matrix	DAT [1)]	TRR Determined by Direct Combustion [mg/kg]	TRR Calculated [2)] [mg/kg]
Phenyl Label			
Forage	20	4.579	4.461
Hay	20	31.697	30.913
Straw	83	18.009	17.315
Chaff	83	1.669	1.555
Grain	83	1.144	1.112

1) DAT = days after treatment
2) TRR was calculated as the sum of ERR + RRR

B. EXTRACTION, CHARACTERISATION AND IDENTIFICATION OF RESIDUES

The extractabilities of ^{14}C-residues from wheat matrices are summarised in Table 6.2/3.

1. Extraction and characterisation of residues in wheat forage

The extractability of the radioactive residues from wheat forage was high with 88.6% of the TRR extracted. Most of the radioactive residues were extracted with methanol (85.2% TRR), while only a minor portion was extracted with water (3.4% of the TRR).
The residual radioactive residues (RRR) after solvent extraction of wheat forage represented portions of 11.4% TRR (0.508 mg/kg).
0.8% of the TRR or 0.033 mg/kg were characterised in the ERR by their HPLC elution behaviour. For characterisation of the RRR of BAS 351 H in wheat forage, the residues remaining after extraction with methanol and water were subjected to a sequential solubilisation procedure, which is described in more details below (see point 11).

2. Extraction and characterisation of residues in wheat hay

The extractability of the radioactive residues from wheat hay was also high with 82.6% of the TRR extracted. The major portion of the radioactive residues was extracted with methanol (73.4% of the TRR). The subsequent water extraction additionally released 9.2% of the TRR.
The residual radioactive residues (RRR) after solvent extraction of wheat hay amounted to 5.378 mg/kg.
1.4% of the TRR or 0.419 mg/kg were characterised in the ERR by their HPLC elution behaviour. For characterisation of the RRR of BAS 351 H in wheat hay, the residues remaining after extraction with methanol and water were subjected to a sequential solubilisation procedure, which is described in more details below (see point 12).

3. Extraction and characterisation of residues in wheat straw

The extractability of the radioactive residues from wheat straw was moderate with 71.5% of the TRR could be extracted. Thereby, portion of 51.6% of the TRR were detected in the methanol extract and a portion of 19.9% of the TRR were measured in the aqueous extract.
The residual radioactive residues (RRR) after solvent extraction of wheat straw accounted for 4.931 mg/kg or 28.5% of the TRR.
14.4% of the TRR or 2.503 mg/kg were characterised in the ERR by their HPLC elution behaviour. For characterisation of the RRR of BAS 351 H in wheat straw, the residues remaining after extraction with methanol and water were subjected to a sequential solubilisation procedure, which is described in more details below (see point 13).

4. Extraction and characterisation of residues in wheat chaff

The extractability of the radioactive residues from wheat chaff was low, 20.5% of the TRR could be extracted. Methanol extraction allowed recovering of 14.1% of the TRR. The succeeding aqueous extraction released a portion of 6.4% of the TRR.
The RRR after solvent extraction of wheat chaff amounted to 1.236 mg/kg or 79.5% of the TRR.
10.8% of the TRR or 0.168 mg/kg were characterised in the ERR by their HPLC elution behaviour. For characterisation of the RRR of BAS 351 H in wheat chaff, the residues remaining after extraction with methanol and water were subjected to a sequential solubilisation procedure, which is described in more details below (see point 14).

5. Extraction and characterisation of residues in wheat grain

In the case of wheat grain, the extractabilities were lowest of all matrices with only 9.5% TRR extracted. The amount of radioactivity extracted with methanol and water was similar with 5.3% of the TRR extracted with methanol and 4.2% of the TRR extracted with water.
The residual radioactive residues (RRR) after solvent extraction of wheat grain amounted to 1.007 mg/kg (or 90.5% of the TRR).
9.3% of the TRR or 0.104 mg/kg were characterised in the ERR by their HPLC elution behaviour. For characterisation of the residual radioactive residues (RRR) of BAS 351 H in wheat grain, the residues remaining after extraction with methanol and water were subjected to a sequential solubilisation procedure, which is described in more details below (see point 15).

Table 6.2/3 Extractability of radioactive residues in wheat samples after foliar treatment with ^{14}C-BAS 351 H

Matrix	DAT[1)]	TRR Calculated[2)]	TRR Combusted	Combined Methanol Extract		Combined Aqueous Extract		ERR[3)]		RRR[4)]	
		[mg/kg]	[mg/kg]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Forage	20	4.461	4.579	3.803	85.2	0.151	3.4	3.954	88.6	0.508	11.4
Hay	20	30.913	31.697	22.695	73.4	2.840	9.2	25.535	82.6	5.378	17.4
Straw	83	17.315	18.009	8.932	51.6	3.451	19.9	12.383	71.5	4.931	28.5
Chaff	83	1.555	1.669	0.219	14.1	0.100	6.4	0.319	20.5	1.236	79.5
Grain	83	1.112	1.144	0.058	5.3	0.047	4.2	0.105	9.5	1.007	90.5

1) DAT = days after last treatment
2) TRR was calculated as the sum of ERR + RRR
3) ERR = extractable radioactive residue
4) RRR = residual radioactive residue (after solvent extraction)

6. Identification and quantification of extractable residues in wheat forage

In wheat forage, the unchanged parent compound bentazone and the *O*-monosaccharide conjugate of a 6-hydroxylated derivative of parent compound (M351H013) formed the major part of the ERR. Additional minor portions of unchanged BAS 351 H and M351H013 were detected in the aqueous ammonia extract and in the macerozyme solubilisate of the RRR. In total, the two compounds were present at amounts of 2.517 mg/kg or 56.4% of the TRR (BAS 351 H) and 1.250 mg/kg or 28.0% of the TRR (M351H013). They were accompanied by several minor metabolites, among them an *O*-monosaccharide conjugate of the 8-hydroxylated derivative of parent compound (M351H017), that co-eluted with the metabolites M351H018, M351H019 (both *N*-disaccharide adducts of parent compound) and M351H020, M351H021 (two monosaccharide conjugates of an aryl-hydroxylated and aryl-methoxylated derivative of parent compound). Additionally, an *N*-monosaccharide conjugate of parent compound (M351H007), co-eluting with the metabolites M351H014, M351H015, M351H016, M351H022 (all *N*-disaccharide adducts of parent compound) was detected. Since these latter minor components were identified in a sample of hay, the composition of the metabolites could be different in forage and other matrices than hay. Therefore, the radioactivity of these metabolites co-eluting in one peak might be represented by only one or all of these compounds. All of these minor metabolites were equal to or below 3% of the TRR in wheat forage.
A summary of the identified components in wheat matrices is given in Table 6.2/4.

7. Identification and quantification of extractable residues in wheat hay

In wheat hay, the unaltered ^{14}C-bentazone and the *O*-glycosylated metabolite M351H013 again represented the major part of the ERR. Again, additional minor portions of unchanged BAS 351 H and M351H013 were detected in the aqueous ammonia extract and in the macerozyme solubilisate of the RRR. In total, the latter compounds were detected at concentrations of 12.068 mg/kg (or 39.0% of the TRR, BAS 351 H) and 12.689 mg/kg (or 41.1% of the TRR, M351H013). The two metabolites were accompanied by minor amounts of M351H0017, M351H018, M351H019, M351H020, M351H021 that were co-eluting in one peak (together 3.0% of the TRR) and the also co-eluting metabolites M351H007, M351H014, M351H015, M351H016, M351H022 (together 3.8% of the TRR).
A summary of the identified components in wheat matrices is given in Table 6.2/4.

8. Identification and quantification of extractable residues in wheat straw

In the methanol and the aqueous extracts of wheat straw, the unchanged parent compound BAS 351 H was the most abundant component; it was also found in the supernatants obtained after aqueous ammonia treatment and enzyme treatment, in total accounting for 8.896 mg/kg or 51.4% of the TRR. Additionally, the metabolites M351H13, the co-eluting metabolites M351H017, M351H018, M351H019, M351H020 and M351H021 and the also co-eluting metabolites M351H007, M351H014, M351H015, M351H016 and M351H022 were detected in minor portions, ranging from 2.9% to 7.0% of the TRR.
A summary of the identified components in wheat matrices is given in Table 6.2/4.

9. Identification and quantification of extractable residues in wheat chaff

In the extracts of wheat chaff, the same metabolites already assigned in the latter matrices were found. Besides the unchanged parent compound (4.2% of the TRR or 0.065 mg/kg) the metabolites M351H013, the co-eluting metabolites M351H0017, M351H018, M351H019, M351H020, M351H021 and the co-eluting metabolites M351H007, M351H014, M351H015, M351H016, M351H022 were detected in minor portions, ranging from 1.0% to 2.6% of the TRR. In addition, one prominent polar component eluting at approximately 6 minutes was detected in the methanol and aqueous extract amounting to ≤0.080 mg/kg or 5.2% of the TRR that was not identified but characterised by its elution behaviour.
A summary of the identified components in wheat matrices is given in Table 6.2/4.

10. Identification and quantification of extractable residues in wheat grain

None of the metabolites identified in the matrices described above was detected in the extractable radioactivity of wheat grain, indicating a limited translocation of the active substance and/or its metabolites after the foliar application. Several minor components and one predominant polar component were detected in the methanol and the aqueous extracts of grain, all of them below 2.6% of the TRR. The unknown polar component was only characterised by its chromatographic properties, but most probably can be assigned to carbohydrates that were also found in the solubilisates of the RRR of grain (see below).

Table 6.2/4 Summary of identified components in wheat matrices after foliar treatment with ^{14}C-BAS 351 H

Metabolite Code	Forage (20 DAT) [1] [mg/kg] ([%TRR])	Hay (20 DAT) [mg/kg] ([%TRR])	Straw (83 DAT) [mg/kg] ([%TRR])	Chaff (83 DAT) [mg/kg] ([%TRR])	Grain (83 DAT) [mg/kg] ([%TRR])
BAS 351 H (M351H000)	2.517 (56.4)	12.068 (39.0)	8.896 (51.4)	0.065 (4.2)	n.d.
M351H013	1.250 (28.0)	12.689 (41.1)	1.217 (7.0)	0.031 (2.1)	n.d.
M351H017, M351H018, M351H019, M351H020, M351H021	0.075 (1.7)	0.928 (3.0)	0.494 (2.9)	0.040 (2.6)	n.d.
M351H007, M351H014, M351H015, M351H016, M351H022	0.136 (3.0)	1.184 (3.8)	0.817 (4.7)	0.016 (1.0)	n.d.
Carbohydrates (glucose)	n.d. [2]	n.d.	n.d.	n.d.	0.647 (58.2)

[1] DAT = days after treatment
[2] n.d. = not detected

Metabolites M351H017, M351H018, M351H019, M351H020, M351H021 and M351H007, M351H014, M351H015, M351H016, M351H022 are co-eluting and were identified after several HPLC clean-up steps from a sample of hay. Therefore, the composition of these components could be different in the other matrices and the radioactivity might be represented by only one or all of these metabolites.

11. Characterisation of the residual radioactive residues after solvent extraction in wheat forage

The first solubilisation step was an extraction with aqueous ammonia solution releasing a minor portion of the radioactive residues (0.100 mg/kg or 2.2% TRR). The remaining residue after aqueous ammonia extraction still contained 0.402 mg/kg (or 9.0% of the TRR) and was subsequently incubated with macerozyme which solubilised a portion of 0.140 mg/kg (or 3.1% of the TRR), a portion of 0.243 mg/kg (or 5.4% of the TRR) still remaining in the residue. The subsequent treatment of the latter residue with tyrosinase/laccase released a portion of 0.049 mg/kg (or 1.1% of the TRR). The following incubation with amylase and amyloglucosidase released another 0.017 mg/kg (or 0.4% of the TRR), while 0.152 mg/kg (or 3.4% of the TRR) were recovered in the final residue.
In total, 0.306 mg/kg (or 6.9% of the TRR) were released during the sequential solubilisation procedure applying treatments with aqueous ammonia and enzymes (see Table 6.2/5).
In summary, 1.1% of the TRR or 0.049 mg/kg were identified in the residues released from RRR (0.6% TRR or 0.029 mg/kg as M351H013 and 0.5% or 0.021 mg/kg as BAS 351 H), and additional 5.2% of the TRR or 0.234 mg/kg were characterised by their HPLC elution behaviour.

12. Characterisation of the residual radioactive residues after solvent extraction in wheat hay

Extraction with aqueous ammonia released a portion of 1.623 mg/kg (5.2% of the TRR) of the radioactive residues. The remaining residue (4.045 mg/kg or 13.1% of the TRR) was then treated with macerozyme, which solubilised additional 1.131 mg/kg (or 3.7% of the TRR). The radioactive residue remaining after this solubilisation step was treated with tyrosinase and laccase which released a further portion of 0.752 mg/kg (or 2.4% of the TRR). The following solubilisation step with amylase and amyloglucosidase released a further portion of 0.218 mg/kg (or 0.7% of the TRR), while 1.644 mg/kg (or 5.3% of the TRR) were still present in the final residue.
In total, 3.725 mg/kg (or 12.0% of the TRR) were released during the sequential solubilisation procedure applying treatments with aqueous ammonia and enzymes (see Table 6.2/5).
In summary, 3.5% of the TRR or 1.089 mg/kg were identified in the residues released from RRR (2.1% TRR or 0.648 mg/kg as M351H013 and 1.4% TRR or 0.441 mg/kg as BAS 351 H) and additional 7.6% of the TRR or 2.363 mg/kg were characterised by their HPLC elution behaviour.

13. Characterisation of the residual radioactive residues after solvent extraction in wheat straw

The initial solubilisation step with aqueous ammonia solution released about a third of the radioactive residues (1.450 mg/kg or 8.4% of the TRR) which may have been incompletely extracted with methanol and water or associated with insoluble plant material (e. g. with proteins). The remaining radioactive residue after aqueous ammonia extraction still contained 3.561 mg/kg (or 20.3% of the TRR) and was subsequently incubated with macerozyme, which solubilised a portion of 1.113 mg/kg (or 6.4% of the TRR). The residue remaining after this solubilisation step was treated with tyrosinase and laccase which released another 0.245 mg/kg (or 1.4% of the TRR). The ensuing incubation of the residue after tyrosinase and laccase treatment with amylase and amyloglucosidase resulted in the release of a portion of 0.078 mg/kg (or 0.5% of the TRR), while 1.886 mg/kg (10.9% of the TRR) remained in the residue.
In total, 2.887 mg/kg (16.7% of the TRR) were released during the sequential solubilisation procedure applying treatments with aqueous ammonia and enzymes (see Table 6.2/5).
Since the radioactivity in the residue was still comparatively high, for further characterisation the residue was treated with solutions of 6 M HCl, 1 M NaOH or was microwave treated. Sodium hydroxide solution released the highest amount of radioactivity from the residue of straw (10.3% of the TRR), while 0.521 mg/kg (3.0% of the TRR) remained in the final residue.
In summary, 4.9% of the TRR or 0.854 mg/kg were identified in the residues released from RRR (1.7% TRR or 0.294 mg/kg as M351H013; 0.3% TRR or 0.050 mg/kg as M351H017-021; 0.4% TRR or 0.067 mg/kg as M351H007, M351H014-016 and M351H022; 2.6% TRR or 0.443 mg/kg as BAS 351 H). Additional 10.0% of the TRR or 1.734 mg/kg were characterised by their HPLC elution behaviour and 10.3% of the TRR were characterised being released after treatment with sodium hydroxide solution.

14. Characterisation of the residual radioactive residues after solvent extraction in wheat chaff

In the first solubilisation step, the residue was extracted with aqueous ammonia which released only a minor part of the radioactive residues (0.045 mg/kg or 2.9% of the TRR). The remaining radioactive residue after aqueous ammonia extraction still contained 1.232 mg/kg (or 79.2% of the TRR) and was subsequently incubated with macerozyme, which solubilised a considerable portion of 0.136 mg/kg or 8.7% of the TRR. The residue remaining after this solubilisation step was treated with tyrosinase and laccase which released only 0.020 mg/kg (or 1.3% of the TRR). The ensuing incubation of the residue after tyrosinase and laccase treatment with amylase and amyloglucosidase resulted in the release of a portion of 0.018 mg/kg (or 1.2% of the TRR), while 0.997 mg/kg (64.1% of the TRR) remained in the residue.
In total, 0.218 mg/kg (14.0% of the TRR) were released during the sequential solubilisation procedure applying treatments with aqueous ammonia and enzymes (see Table 6.2/5).
Since the residue level still was comparatively high, for further characterisation the residue was treated with solutions of 6 M HCl, 1 M NaOH or was microwave treated. Sodium hydroxide solution released the highest amount of radioactivity from the residue of chaff (31.8% of the TRR), while 0.482 mg/kg (31.0% of the TRR) remained in the final residue.
In summary, 0.7% of the TRR or 0.011 mg/kg were identified in the residues released from RRR (0.3% TRR or 0.004 mg/kg as M351H013 and 0.4% TRR or 0.006 mg/kg as M351H017-021) and 11.5% of the TRR or 0.178 mg/kg were characterised by their HPLC elution behaviour. Another 31.8% of the TRR were characterised by solubilisation with sodium hydroxide solution.

15. Characterisation of the residual radioactive residues after solvent extraction in wheat grain

High radioactivity levels (90.5% of the TRR) were found in the residual radioactive residues after solvent extraction. During the subsequent sequential solubilisation procedure with aqueous ammonia and enzymes altogether 75.4% of the TRR was solubilised from the RRR (see Table 6.2/5). In the case of grain the most effective solubilisation was achieved by incubation of the residual radioactive residues with macerozyme (44.5% TRR) and amylase/amyloglucosidase (12.8% TRR). The main component detected in the supernatants after enzyme treatment was identified as carbohydrates (glucose), altogether amounting to 0.647 mg/kg or 58.2% of the TRR (see Table 6.2/4). Each of the other components detected in the solubilisates of the RRR of grain were below 1.9% of the TRR.

Table 6.2/5 Characterisation of the residual radioactive residues (RRRs)

Fraction / Solubilisate	Crop Matrix									
	Wheat Forage		Wheat Hay		Wheat Straw		Wheat Chaff		Wheat Grain	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
RRR	0.508	11.4	5.378	17.4	4.931	28.5	1.236	79.5	1.007	90.5
NH_4OH solubilsate	0.100	2.2	1.623	5.2	1.450	8.4	0.045	2.9	0.097	8.7
NH_4OH residue	0.402	9.0	4.045	13.1	3.516	20.3	1.232	79.2	0.906	81.4
Glucosidase / Hesperidinase supernatant	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	0.104	9.4
Glucosidase / Hesperidinase residue	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	0.763	68.6
Macerozyme supernatant	0.140	3.1	1.131	3.7	1.113	6.4	0.136	8.7	0.495	44.5
Macerozyme residue	0.243	5.4	2.806	9.1	2.184	12.6	1.041	66.9	0.257	23.1
Tyrosinase / Laccase supernatant	0.049	1.1	0.752	2.4	0.245	1.4	0.020	1.3	n. a.	n. a.
Tyrosinase / Laccase residue	0.174	3.9	1.981	6.4	1.909	11.0	1.012	65.0	n. a.	n. a.
Amylase / Amyloglucosidase supernatant	0.017	0.4	0.218	0.7	0.078	0.5	0.018	1.2	0.143	12.8
Amylase / Amyloglucosidase residue	0.152	3.4	1.644	5.3	1.886	10.9	0.997	64.1	0.100	9.0
Sum of solubilised radioactive residues	**0.306**	**6.9**	**3.725**	**12.0**	**2.887**	**16.7**	**0.218**	**14.0**	**0.838**	**75.4**
Final residue [1)]	0.152	3.4	1.644	5.3	1.886	10.9	0.997	64.1	0.100	9.0
Procedural recovery [%]	**90.3**		**99.8**		**96.8**		**98.3**		**93.2**	
6 M HCl solubilisate	n. a.	n. a.	n. a.	n. a.	0.510	2.9	0.447	28.7	0.041	3.7
6 M HCl residue	n. a.	n. a.	n. a.	n. a.	1.304	7.5	0.482	31.0	0.051	4.6
1 M NaOH solubilisate	n. a.	n. a.	n. a.	n. a.	1.790	10.3	0.494	31.8	0.081	7.2
1 M NaOH residue	n. a.	n. a.	n. a.	n. a.	0.521	3.0	0.482	31.0	0.028	2.5
Microwave Extraction solubilisate	n. a.	n. a.	n. a.	n. a.	0.743	4.3	0.311	20.0	0.051	4.6
Microwave Extraction residue	n. a.	n. a.	n. a.	n. a.	1.283	7.4	0.675	43.4	0.052	4.7

[1)] For wheat straw, chaff and grain additional solubilisation steps were performed. In all cases sodium hydroxide solution released the highest amount of radioactivity from the respective residue. Therefore the radioactivity values of the residue after sodium hydroxide solubilisation are cited as the final residue for these matrices.

16. Proposed metabolic pathway

The parent compound was found to be moderately metabolised until harvest. Portions between approximately 39% and 56% of the total radioactive residues were still present as unchanged active substance BAS 351 H in forage, hay and straw. The major metabolite in quantitative terms was an *O*-monosaccharide conjugate of 6-hydroxylated derivative of parent compound (M351H013). Further components were detected at minor amounts. They were also generated by hydroxylation of the benzothiadiazine ring and subsequent conjugation with monosaccharide or with a methyl group (see Figure 6.2/2). In addition, the benzothiadiazine ring was conjugated with monosaccharide / disaccharide, in which the sugar moiety is directly attached to the free sulfonamide nitrogen. Decreasing extractability of the harvest material indicated that the transformation products were also incorporated into or associated with insoluble cell wall polymers and starch. In addition, radiolabelled carbohydrates (glucose) were found, particularly in grain, which results from complete degradation of the phenyl ring into compounds suitable for the biosynthesis of carbohydrates and re-assimilation into natural compounds.

17. Storage stability of residues

A comparison of the extractability and of the HPLC chromatograms of the metabolite patterns obtained at the beginning and at the end of the investigation period showed that there was no relevant change in the nature of radioactive residues during sample storage over a period of approximately 17 months in the extract and about 21 months in matrix.

III. CONCLUSION

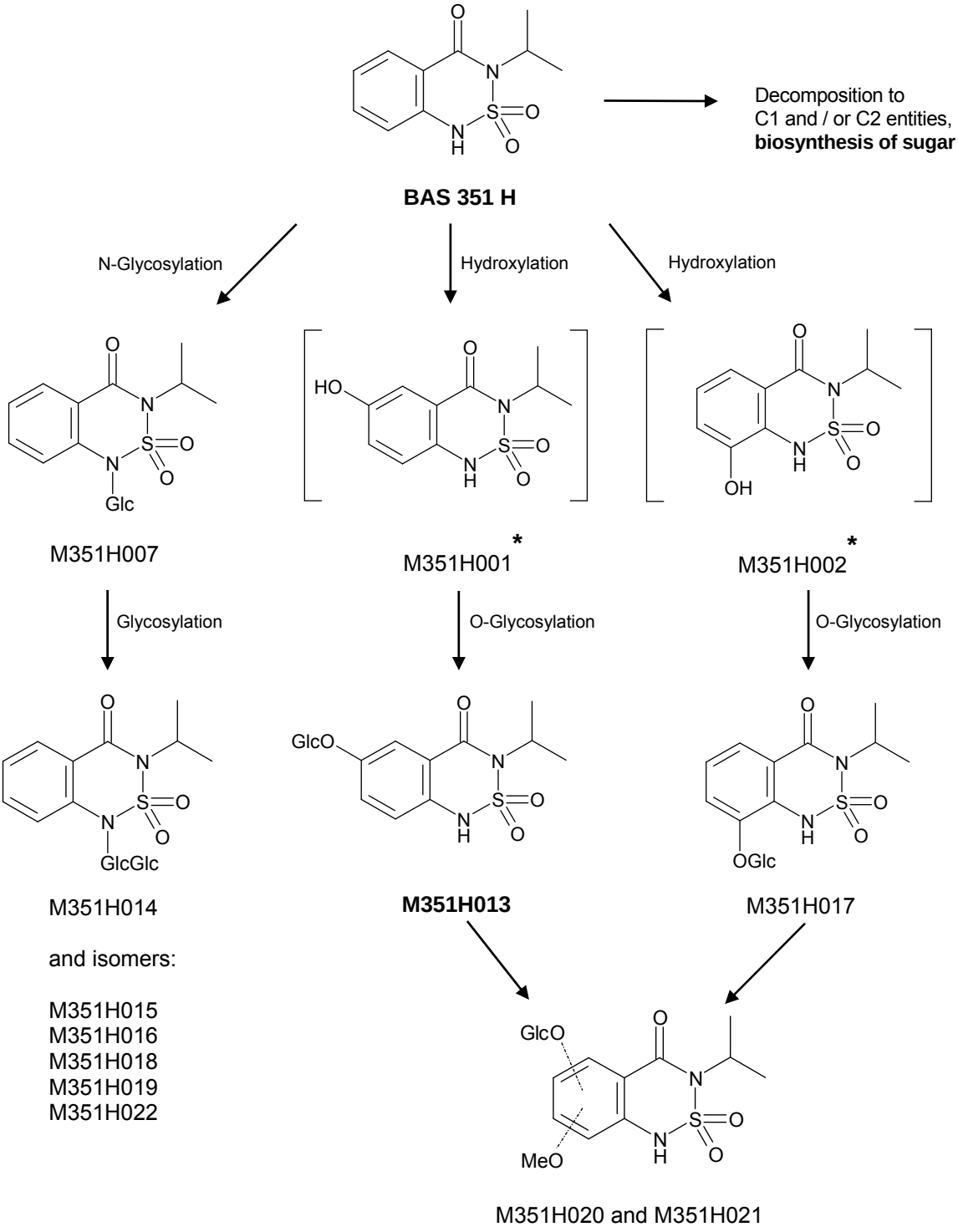
The wheat metabolism study was conducted with ^{14}C-BAS 351 H, which was applied once to the plants at a nominal rate of 1 kg a.s./ha. The highest levels of total radioactive residues (TRR) were found in wheat hay (30.913 mg/kg) followed by straw (17.315 mg/kg) and forage (4.461 mg/kg). The total radioactive residues were lowest in chaff (1.555 mg/kg) and grain (1.112 mg/kg).

The extractability of radioactive residues with methanol and water was high for wheat forage (> 88% of the TRR). From wheat hay and wheat straw portions of 82.6% and 71.5% of the TRR were extracted. For wheat chaff and grain the extractability of the radioactive residues was considerably lower with portions of 20.5% and 9.5% of the TRR extracted. For all matrices, extractability was very high for bentazone and its conjugated 6-OH and 8-OH-metabolites.

The parent compound was found to be incompletely metabolised until harvest during the investigation of the nature of the residue. BAS 351 H was identified in wheat forage, hay and straw with 56.4%, 39.0% and 51.4% of the TRR. The major metabolite in forage and hay was identified as the *O*-monosaccharide conjugate of a 6-hydroxylated derivative of parent compound (M351H0013) amounting to 28.0% and 41.1% of the TRR. In straw and chaff M351H013 was detected at lower amounts (≤ 7.0% of the TRR). Further components detected were a *O*-monosaccharide conjugate of 8-hydroxylated derivative of parent compound (M351H0017), two monosaccharide conjugates of an aryl-hydroxylated and aryl-methoxylated derivative of parent compound (M351H020, M351H021) and a *N*-monosaccharide conjugate of the parent compound (M351H007) as well as some *N*-disaccharide adducts of parent compound, all ≤ 5% of the TRR.
In grain, the main component detected in the supernatants after enzyme treatments was assigned to carbohydrates (glucose), amounting to about 58% of the TRR.

Based on the identified metabolites the proposed metabolic pathway of bentazone in wheat involves the hydroxylation of the benzothiadiazine ring. The hydroxyl groups were subsequently glycosylated generating the metabolites M351H013 and M351H017. In addition, the benzothiadiazine ring was conjugated with monosaccharide/disaccharide at the nitrogen atom at position one. Further metabolism lead to the degradation of the carbon skeleton of bentazone and re-assimilation into natural compounds like carbohydrates.

Figure 6.2/2 Proposed metabolic pathway of bentazone (BAS 351 H) in wheat



* not detected as free metabolites in wheat matrices

6.2.2 Poultry

An overall of six livestock metabolism studies (3 goat and 3 hen) were conducted with bentazone and its 6-OH- and 8-OH-metabolites, respectively, all of which were considered appropriate in the initial evaluation. An overview of all metabolites including structures, names, codes and synonyms can be found in Tab 6.11/1 (M-II, 6.11).

Goat and poultry studies with bentazone and its 6-OH- and 8-OH-metabolites showed minimal transfer of residues to meat, fat, edible organs, milk and eggs. Therefore, consumer exposure to residues in food from animal origin is not anticipated when the product is used as recommended.
No additional metabolism studies were necessary to estimate the fate of residues in livestock. Nevertheless, to verify results from these originally submitted animal metabolism studies, a rat metabolism study was conducted and is also submitted with this dossier (a summary can be found in Doc M-II, 5.1). In this rat metabolism study all previously reported metabolites from animal origin were found and identified with state-of-the-art analytical techniques. A summary of initially submitted livestock metabolism studies is given in the paragraph below.

Bentazone was predominately rapidly excreted *via* urine (average >85%), mainly as unchanged parent and small amounts of 6-OH- and 8-OH-bentazone when fed to goats. Feeding of 6-OH- and 8-OH-bentazone to goats revealed faster excretion compared to parent bentazone. Only minor metabolites were detected in milk or edible tissues in all three studies.
Metabolism studies with bentazone and its 6-OH- and 8-OH-metabolites in laying hens showed a similar metabolic pathway and excretion behaviour compared to goats and rats.

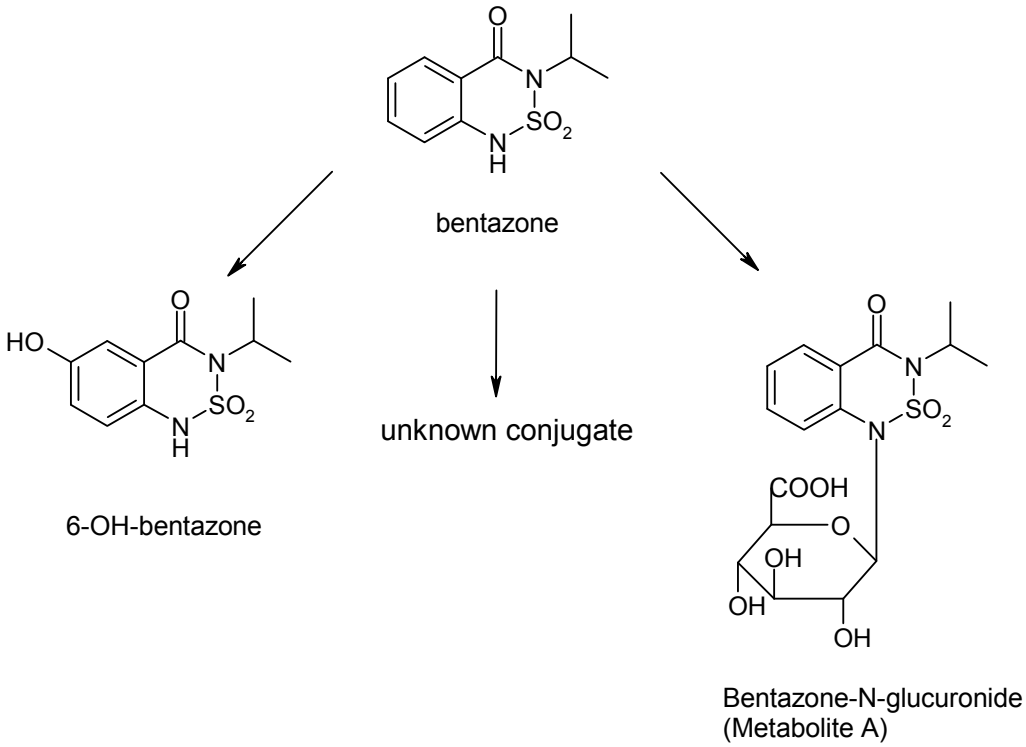
The metabolism and distribution in livestock of bentazone was investigated using ^{14}C-bentazone, ^{14}C-6-OH-bentazone and ^{14}C-8-OH-bentazone. In summary, the metabolic pathway of bentazone in livestock includes *N*-glucuronidation as well as oxidation to 6-OH-bentazone with subsequent conjugation. Both 6-OH- and 8-OH-bentazone are excreted either directly or *via* conjugates, including the sulphate in case of 6-OH-bentazone.

In laying hens treated with ^{14}C-bentazone, 72% of the dose, consisting of 45% bentazone, 15% 6-OH-bentazone and 12% bentazone-*N*-glucuronide (Metabolite A, M351H004) were found in excreta. The highest concentration of radioactivity in tissues was determined in kidney. The major portion of the radioactivity in extract of liver, muscle, fat and eggs was associated with bentazone and Metabolite A (M351H004).
After ^{14}C-6-OH-bentazone treatment, 42% and 45% of the dose were detected in excreta before and after treatment with β-glucuronidase/sulphatase, respectively, consisting of a compound with the same HPLC retention time as 6-OH-bentazone and a more polar fraction which is probably at least partly a conjugate of 6-OH-bentazone. The highest concentration of radioactivity in tissues was observed in kidney and liver. The only component above the limit of detection in extracts of liver was associated with the polar fraction detected in excreta. Concentration of radioactivity in other tissues was too low for investigation of metabolites.
After ^{14}C-8-OH-bentazone treatment, 67% and 88% of the dose were detected in excreta before and after treatment with β-glucuronidase/sulphatase, respectively, consisting of a compound with the same HPLC retention time as 8-OH-bentazone and a more polar fraction which is probably a conjugate of 8-OH-bentazone. The only component above the limit of detection in extracts of liver was associated with the polar fraction detected in excreta. Concentration of radioactivity in other tissues was too low for investigation of metabolites.

In lactating goats treated with ^{14}C-bentazone, the majority of radioactivity was excreted *via* urine and faeces. The highest concentration of radioactivity in tissues was determined in fat and kidney. Milk, tissue and faeces contained unchanged bentazone. In liver of one goat, 11% TRR was identified as bentazone-*N*-glucuronide. In urine, unchanged bentazone and small amounts of 6-OH- and 8-OH-bentazone (0.2% TRR each) were found.
After ^{14}C-6-OH-bentazone treatment, the highest concentration of radioactivity in tissues was determined in kidney. 6-OH-bentazone was identified in fat, kidney, muscle and liver (94%, 73%, 44% and 44% TRR, respectively). The percentage of conjugates amounted to 5%, 7% and 35% in kidney, muscle and liver, respectively. The liver conjugate was identified as the sulphate of 6-OH-bentazone. Milk showed no 6-OH-bentazone but up to four other metabolites. The major metabolite with about 72% of the extractables was identified as the sulphate. Unidentified metabolites accounted for 22% TRR each in kidney and muscle. The radioactivity in urine and faeces consisted mainly of 6-OH-bentazone. In urine conjugates accounted for about 10% TRR and the major conjugate was identified as the sulphate of 6-OH-bentazone. Bile contained no 6-OH-bentazone but yielded two additional polar fractions of 19% and 74% TRR. The major one was identified as conjugate.
After ^{14}C-8-OH-bentazone treatment, the highest concentration of radioactivity in tissues was determined in kidney. Residues in milk, muscle, fat, liver and kidney could be ascribed to unchanged 8-OH-bentazone (29%, 61%, 82%, 75% and 95% TRR, respectively) and conjugated thereof (41%, 21%, 5%, 11% and 3%, respectively). The sum of unknown metabolites and losses amounted to 2-30% TRR. The radioactivity in urine and faeces consisted exclusively of 8-OH-bentazone, while bile contained 8-OH-bentazone and 62% of conjugates.

Due to their polar nature bentazone and its metabolites 6-OH- and 8-OH-bentazone are excreted rather than accumulated in tissue, milk and eggs. Therefore, consumer exposure to residues in food from animal origin is not anticipated when the product is used as recommended.

Figure 6.2/3 Metabolic pathway of bentazone in livestock (goat and hen)



6.2.3 Lactating ruminants (goat or cow)

The relevant information is given in the previous chapter (see Doc M-II, 6.2.2) as an overall summary of livestock metabolism.

6.2.4 Pigs

No metabolism study in pigs was performed since the metabolite patterns in rodents (rats) and ruminants (goats) did not differ significantly.

6.2.5 Nature of residue in fish

Due to the very low $P_{o/w}$ value of bentazone, no bioaccumulation study is required.

6.3 Residue trials (supervised field trials)

A large amount of residue data was submitted with the Annex II Dossier (1995) for Annex I inclusion. These data are not reported here again. A number of new residue studies have been performed to complete the original data sets for MRL proposals (see chapter 6.7). In addition, a distinction between fresh a dry peas is made, as requested in the Full Report (1998).

6.3.1 Root and tuber vegetables

Potatoes

Table 6.3/1 GAP for the use of BAS 351 H in/on potatoes

Crop	Maximum Applied Dose	Water Volume	PHI	Application Method	Application Timing
Potato	1 x 0.96 kg BAS 351 H/ha	200-400 L/ha	F	spray application	BBCH 13-19

PHI = pre-harvest interval

Table 6.3/2 GAP information of residue trials conducted in potatoes in 2009

Region	Country	Formulation	Application[0]				DALA[1]
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
EU South	Spain (2 trials)	BAS 351 45 H, SG	spray application	0.957	0.479	1	0 56-104
	Italy (2 trials)						
	France (2 trials)						
	Greece (2 trials)						

0) actual application rates varied by 10% at most
1) days after last application

Table 6.3/3 GAP information of residue trials conducted in potatoes before 2009 already reviewed in the context of Annex I inclusion

Region Doc ID	Country (Trial No.)	Formulation	Application[0]				DALA[1]	Total Bentazone Residues (mg/kg)
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.		
Northern Europe 1994/10626	Switzerland (35107H78/10E)	BAS 351 07 H	n.r.	0.96	n.r.	1	140	<0.06
	Switzerland (35107H78/12E)	BAS 351 07 H	n.r.	0.96	n.r.	1	140	<0.06
	Switzerland (35107H78/8E)	BAS 351 07 H	n.r.	0.96	n.r.	1	140	<0.06
	Germany (35107H79/13A)	BAS 351 07 H	n.r.	0.96	0.240	1	68 75 89	n.rel. n.rel. <0.06
	Germany (35107H79/14A)	BAS 351 07 H	n.r.	0.96	0.291	1	58 67 81	n.rel. n.rel. <0.06
	Germany (35107H79/15A)	BAS 351 07 H	n.r.	0.96	0.240	1	42 49 63	n.rel. n.rel. <0.06
	Germany (35107H79/16A)	BAS 351 07 H	n.r.	0.96	0.240	1	89 97 111	n.rel. n.rel. 0.06
	Sweden (35107H76/11E)	BAS 351 07 H	n.r.	1.2	0.200	1	121	<0.06
	Sweden (35107H76/12E)	BAS 351 07 H	n.r.	1.2	0.200	1	117	<0.06
	Sweden (35107H76/13E)	BAS 351 07 H	n.r.	1.2	0.200	1	104	<0.06
	Germany (35132H86/1A)	BAS 351 32 H	n.r.	0.96	0.240	1	1 8 14 21 28	n.rel. n.rel. n.rel. n.rel. <0.06
	Germany (35132H86/2A)	BAS 351 32 H	n.r.	0.96	0.240	1	1 8 14 21 28	n.rel. n.rel. n.rel. n.rel. <0.06
	Germany (35132H86/3A)	BAS 351 32 H	n.r.	0.96	0.240	1	1 8 14 21 28	n.rel. n.rel. n.rel. n.rel. <0.06

0) actual application rates varied by 10% at most
1) days after last application
n.r. not reported
n.rel. not relevant

Report:	II A 6.3.1/1 Schroth E., Martin T. 2010(f) Study on the residue behavior of BAS 351 H (Bentazone) in potato after the application of BAS 351 45 H under field conditions in France (South), Greece, Italy and Spain, 2009 BASF DocID 2010/1144246
Guidelines:	EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8); EEC 1607/VI/97 rev. 2 10.06.1999; EEC 7029/VI/95 rev. 5; EEC 7525/VI/95 rev. 7
GLP:	Yes (laboratory certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

Executive Summary

During the 2009 growing season, 8 trials in potato were conducted in different representative growing areas in Southern Europe to determine the residue level of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC). BAS 351 45 H (87% bentazone, SG) was applied once at a rate equivalent to 0.957 kg a.s./ha of bentazone. The application was done at crop height 5-15 cm. Specimens of whole plant without roots were collected at the day of the application. Specimens of potato tubers were collected at BBCH 49 (harvest).
The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. Total bentazone residues in potato tuber specimens at BBCH 49 were between <0.03 and 0.08 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** BAS 351 45 H (SG)
   Description: Basagran (SL)
   Lot/Batch #: 2002-1; 87% bentazone nominal
   Purity: Not reported
   CAS#: 25057-89-0
   Development code: Not reported
   Spiking levels: 0.01-80 mg/kg

2. **Test Commodity:**
   Crop: Potato
   Type: Root and tuber vegetables
   Variety: Carlita, Finca, Agatha, Spunta, Mona Lisa, Carrera
   Botanical name: *Solanum tuberosum*
   Crop parts(s) or processed commodity: Tubers, whole plants without roots
   Sample size: 0.5-2.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2009 growing season, 8 trials in potato were conducted in different representative growing areas in France (South), Greece, Italy and Spain to determine the residue level of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
BAS 351 45 H (87% bentazone, SG) was applied once at a rate equivalent to 0.957 kg a.s./ha of bentazone and the spray volume used was 200 L/ha. The application was done at crop height 5-15 cm.
Specimens of whole plant without roots were collected at the day of the application. Specimens of potato tubers were collected at BBCH 49 (harvest). Untreated control specimens were taken at every time point. Specimens were stored frozen (≤-18°C) until analysis. The maximum storage interval from harvest until analysis was 367 days.

Table 6.3/4 Target application rates and timings for potatoes

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2009	8	1	F	BAS 351 45 H (SG)	BAS 351 H	0.957	200	at 5-15 cm crop height

2. Description of analytical procedures

The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg.
Samples were extracted with aqueous methanol, followed by iso-octane extraction and liquid/liquid partition of an aliquot. Hydrolysis was performed using enzymatic cleavage, and a $Ca(OH)_2$-precipitation step was included to remove acidic matrix constituents. Reversed phase C_{18}-SPE clean-up was performed before the final determination by liquid chromatography and tandem mass spectrometric detection (LC-MS/MS).

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3/5, detailed residue levels are shown in Table 6.3/6.
The bentazone residues in potato whole plant (no roots) specimens taken directly after the application ranged between 31-81 mg/kg. At BBCH 49, residues in tuber ranged between <0.01-0.06 mg/kg.
The 6-OH-bentazone residues in potato whole plant (no roots) specimens taken directly after the application ranged between 0.50-5.7 mg/kg. At BBCH 49, residues in tuber were all below the LOQ (<0.01 mg/kg).
The 8-OH-bentazone residues in potato whole plant (no roots) specimens taken directly after the application ranged between 0.57-1.6 mg/kg. At BBCH 49, residues in tuber were all below the LOQ (<0.01 mg/kg).
Total bentazone residues in potato whole plant (no roots) specimens taken directly after the application ranged between 32.33-82.77 mg/kg. At BBCH 49, residues in tuber were between <0.03-0.08 mg/kg.
No residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone above the limit of quantitation (0.01 mg/kg) were found in the control specimens of this study.

Table 6.3/5 Summary of residues in potatoes

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU South	2009	0	09-17	whole plant[5]	31-81	0.5-5.7	0.57-1.6	32.33-82.77
		56-104	**49**	**tuber**	**<0.01-0.06**	**<0.01**	**<0.01**	**<0.03-0.08**

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) without roots

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Total bentazone residues in potato tuber specimens at BBCH 49 were between <0.03-0.08 mg/kg.

Table 6.3/6 Residues of bentazone in potato after one application of BAS 351 45 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	370247	Potato	Spain	BAS 351 45 H: 1 x 0.957	15	0	plant[5]	53	0.65	1.6	55.11	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2010/1144246					61	tuber	<0.01	<0.01	<0.01	<u>≤0.03</u>	Bentazone				
Trial No.	L090388											w. plant[5]	0.01-80	3	105	1.1
GLP:	yes											tuber	0.01-0.10	4	105	5.2
Year	2009											6-OH-bentazone				
Study code:	370247	Potato	Italy	BAS 351 45 H: 1 x 0.957	09-15	0	plant[5]	36	1.0	0.87	37.76	w. plant[5]	0.01-10	3	105	2.5
Doc ID:	2010/1144246					104	tuber	0.01	<0.01	<0.01	<u>0.03</u>	tuber	0.01-0.10	4	94	4.9
Trial No.	L090389											8-OH-bentazone				
GLP:	yes											w. plant[5]	0.01-4	3	90	10
Year	2009											tuber	0.01-0.10	4	95	4.9
Study code:	370247	Potato	France (South)	BAS 351 45 H: 1 x 0.957	11	0	plant[5]	62	1.8	1.6	65.19					
Doc ID:	2010/1144246					75	tuber	0.02	<0.01	<0.01	<u>0.04</u>					
Trial No.	L090390															
GLP:	yes															
Year	2009															
Study code:	370247	Potato	Greece	BAS 351 45 H: 1 x 0.957	13-17	0	plant[5]	60	3.1	0.87	63.73					
Doc ID:	2010/1144246					56	tuber	0.02	<0.01	<0.01	<u>0.04</u>					
Trial No.	L090391															
GLP:	yes															
Year	2009															
Study code:	370247	Potato	Spain	BAS 351 45 H: 1 x 0.957	15	0	plant[5]	35	0.50	0.89	36.30					
Doc ID:	2010/1144246					63	tubers	0.06	<0.01	<0.01	<u>0.08</u>					
Trial No.	L090392															
GLP:	yes															
Year	2009															

Table 6.3/6 Residues of bentazone in potato after one application of BAS 351 45 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	370247	Potato	Italy	BAS 351 45 H: 1 x 0.957	09-15	0	plant[5]	71	5.7	1.0	77.29					
Doc ID:	2010/1144246					73	tubers	<0.01	<0.01	<0.01	<u>≤0.03</u>					
Trial No.	L090393															
GLP:	yes															
Year	2009															
Study code:	370247	Potato	France (South)	BAS 351 45 H: 1 x 0.957	19	0	plant[5]	81	0.68	1.2	82.77					
Doc ID:	2010/1144246					74	tubers	<0.01	<0.01	<0.01	<u>≤0.03</u>					
Trial No.	L090394															
GLP:	yes															
Year	2009															
Study code:	370247	Potato	Greece	BAS 351 45 H: 1 x 0.957	13	0	plant[5]	31	0.85	0.57	32.33					
Doc ID:	2010/1144246					94	tubers	<0.01	<0.01	<0.01	<u>≤0.03</u>					
Trial No.	L090395															
GLP:	yes															
Year	2009															

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) at application
_ underlined values were used for MRL calculation

6.3.2 Bulb vegetables

Table 6.3/7 GAP for the use of BAS 351 H in/on onions

Crop	Maximum Applied Dose	Water Volume	PHI	Application Method	Application Timing
Bulb onion Spring onion	1 x 0.96 kg BAS 351 H/ha	200-400 L/ha	F	spray application	BBCH 12-14

PHI = pre-harvest interval

Table 6.3/8 GAP information of residue trials conducted in bulb onions between 1999 and 2009

Region	Country	Formulation	Application[0]				DALA[1]
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
EU North	The Netherlands (1 trial)	BAS 351 32 H, SG	spray application	1.55	0.517	1	28-39 42-56 85-100
	France (1 trial)						
	Germany (2 trials)						
EU South	Spain (2 trials)	BAS 351 45 H, SG	spray application	0.957	0.479	1	0 29-30 40-44
	Italy (2 trials)						
	Greece (2 trials)						
	France (2 trials)						

0) actual application rates varied by 10% at most
1) days after last application

Table 6.3/9 GAP information of residue trials conducted in spring onions between 1999 and 2009

Region	Country	Formulation	Application[0]				DALA[1]
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
EU North	Germany (1 trial)	BAS 351 45 H, SG	spray application	0.722	0.361	1	0 23-24
	The Netherlands (1 trial)						
EU South	Spain (1 trial)	BAS 351 45 H, SG	spray application	0.957	0.479	1	0 30
	Greece (1 trial)						

0) actual application rates varied by 10% at most
1) days after last application

Bulb Onions

Report: II A 6.3.2/1
Schroth E., Martin T. 2010(g)
Study on the residue behavior of BAS 351 H (Bentazone) in bulb onions after the application of BAS 351 45 H under field conditions in France (South), Greece, Italy and Spain, 2009
BASF DocID 2010/1164276

Guidelines: EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8); EEC 1607/VI/97 rev. 2 10.06.1999; EEC 7029/VI/95 rev. 5; EEC 7525/VI/95 rev. 7

GLP: Yes
(laboratory certified by ENAC, Entidad Nacional de Acreditación, Madrid, Spain)

Executive Summary

During the 2009 growing season, 8 trials in bulb onions were conducted in different representative growing areas in Southern Europe to determine the residue level of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC). BAS 351 45 H (87% bentazone, SG) was applied once at a rate equivalent to 0.957 kg a.s./ha of bentazone. The application was done at 30±1 days before harvest. Specimens of whole plant without roots were collected at the day of the application. Specimens of bulb onions were collected about 30 days after the application. In case of immaturity an additional sampling of bulbs was done at growth stage BBCH 49.
The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. Total bentazone residues in onion bulb specimens at DALA 29-30 (BBCH 45-49) were between <0.03-0.04 mg/kg and at BBCH 49 below 0.03 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 45 H (SG)
Lot/Batch #:	2002-1, 87% bentazone, nominal
Purity:	Not reported
CAS#:	25057-89-0
Development code:	Not reported
Spiking levels:	0.01-10 mg/kg

2. Test Commodity:

Crop:	Onion
Type:	Bulb vegetables
Variety:	Tardia de Lerida, Dorata di Bologna, Sturon, Glacier, Valencia Tardia, Densoty, Banko 936, Doree de Parme
Botanical name:	*Allium cepa*
Crop parts(s) or processed commodity:	Bulbs, whole plants without roots
Sample size:	0.5-2.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2009 growing season, 8 trials in bulb onions were conducted in different representative growing areas in France (South), Greece, Italy and Spain to determine the residue level of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
BAS 351 45 H (87% bentazone, SG) was applied once at a rate equivalent to 0.957 kg a.s./ha of bentazone and the spray volume used was 200 L/ha. The application was done at 30±1 days before harvest.
Specimens of whole plant without roots were collected at the day of the application. Specimens of bulb onions were collected about 30 days after the application. In case of immaturity an additional sampling of bulbs was done at growth stage BBCH 49. Untreated control specimens were taken at every time point. Specimens were stored frozen (≤-18°C) until analysis. The maximum storage interval from harvest until analysis was 321 days.

Table 6.3/10 Target application rates and timings for bulb onions

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2009	8	1	F	BAS 351 45 H (SG)	BAS 351 H	0.957	200	30±1 days before harvest

2. Description of analytical procedures

The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg.
Samples were extracted with aqueous methanol, followed by iso-octane extraction and liquid/liquid partition of an aliquot. Hydrolysis was performed using enzymatic cleavage, and a $Ca(OH)_2$-precipitation step was included to remove acidic matrix constituents. Reversed phase C_{18}-SPE clean-up was performed before the final determination by liquid chromatography and tandem mass spectrometric detection (LC-MS/MS).

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3/11, detailed residue levels are shown in Table 6.3/12.
The bentazone residues in bulb onions whole plant no roots specimens taken directly after the application ranged between 3.7-9.0 mg/kg. At 29-30 DALA the residues in bulbs had declined to levels between <0.01-0.02 mg/kg. At 40-44 DALA (BBCH 49), residues in bulbs were all below the LOO (<0.01 mg/kg).
The 6-OH-bentazone residues in bulb onions whole plant no roots specimens taken directly after the application ranged between 0.01-0.05 mg/kg. At 29-30 DALA and also at 40-44 DALA (BBCH 49), residues in bulbs were all below the LOQ (<0.01 mg/kg).
The 8-OH-bentazone residues in bulb onions whole plant no roots specimens taken directly after the application ranged between 0.02-0.06 mg/kg. At 29-30 DALA and also at 40-44 DALA (BBCH 49), residues in bulbs were all below the LOQ (<0.01 mg/kg).
The total bentazone residues in bulb onions whole plant no roots specimens taken directly after the application ranged between 3.73-9.07 mg/kg. At 29-30 DALA the residues in bulbs had declined to levels between <0.03-0.04 mg/kg. At 40-44 DALA (BBCH 49), residues in bulbs were all below 0.03 mg/kg.
No residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone above the limit of quantitation (0.01 mg/kg) were found in the control specimens of this study.

Table 6.3/11 Summary of residues in bulb onions

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU South	2009	0	41-43	whole plant[5]	3.7-9.0	0.01-0.05	0.02-0.06	3.73-9.07
		29-30	**45-49**	**bulb**	**<0.01-0.02**	**<0.01**	**<0.01**	**<0.03-0.04**
		40-44	**49**	**bulb**	**<0.01**	**<0.01**	**<0.01**	**<0.03**

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) without roots

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Total bentazone residues in onion bulb specimens were between <0.03-0.04 mg/kg at DALA 29-30 (BBCH 45-49) and below 0.03 mg/kg at BBCH 49.

Table 6.3/12 Residues of bentazone in bulb onion after one application of BAS 351 45 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	370248	Onion	Spain	BAS 351 45 H: 1 x 0.957	43	0	plant[5]	5.0	0.02	0.04	5.06	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2010/1164276					30	bulb	<0.01	<0.01	<0.01	<u>≤0.03</u>	Bentazone				
Trial No.	L090370											w. plant[5]	0.01-10	3	103	5.0
GLP:	yes											bulb	0.01-0.10	4	101	8.8
Year	2009											6-OH-bentazone				
Study code:	370248	Onion	Italy	BAS 351 45 H: 1 x 0.957	43	0	plant[5]	3.7	0.01	0.02	3.73	w. plant[5]	0.01-0.1	2	92	N/A
Doc ID:	2010/1164276					29	bulb	<0.01	<0.01	<0.01	<u>≤0.03</u>	bulb	0.01-0.10	4	99	8.4
Trial No.	L090371					43	bulb	<0.01	<0.01	<0.01	<0.03	8-OH-bentazone				
GLP:	yes											w. plant[5]	0.01-0.1	2	93	N/A
Year	2009											bulb	0.01-0.10	4	96	6.4
Study code:	370248	Onion	Greece	BAS 351 45 H: 1 x 0.957	45	0	plant[5]	7.8	0.03	0.06	7.89					
Doc ID:	2010/1164276					30	bulb	0.02	<0.01	<0.01	<u>0.04</u>					
Trial No.	L090372															
GLP:	yes															
Year	2009															
Study code:	370248	Onion	France (South)	BAS 351 45 H: 1 x 0.957	43	0	plant[5]	4.3	0.02	0.06	4.38					
Doc ID:	2010/1164276					29	bulb	<0.01	<0.01	<0.01	<u>≤0.03</u>					
Trial No.	L090373															
GLP:	yes															
Year	2009															
Study code:	370248	Onion	Spain	BAS 351 45 H: 1 x 0.957	43	0	plant[5]	9.0	0.03	0.04	9.07					
Doc ID:	2010/1164276					30	bulb	<0.01	<0.01	<0.01	<u>≤0.03</u>					
Trial No.	L090374															
GLP:	yes															
Year	2009															

Table 6.3/12 Residues of bentazone in bulb onion after one application of BAS 351 45 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	370248	Onion	Italy	BAS 351 45 H: 1 x 0.957	43-45	0	plant[5]	4.2	0.05	0.02	4.27					
Doc ID:	2010/1164276					30	bulb	<0.01	<0.01	<0.01	<u>0.03</u>					
Trial No.	L090375					40	bulb	<0.01	<0.01	<0.01	<0.03					
GLP:	yes															
Year	2009															
Study code:	370248	Onion	Greece	BAS 351 45 H: 1 x 0.957	41	0	plant[5]	7.4	0.04	0.04	7.48					
Doc ID:	2010/1164276					30	bulb	<0.01	<0.01	<0.01	<u>0.03</u>					
Trial No.	L090376					44	bulb	<0.01	<0.01	<0.01	<0.03					
GLP:	yes															
Year	2009															
Study code:	370248	Onion	France (South)	BAS 351 45 H: 1 x 0.957	43	0	plant[5]	3.8	0.02	0.03	3.85					
Doc ID:	2010/1164276					29	bulb	<0.01	<0.01	<0.01	<u>0.03</u>					
Trial No.	L090377															
GLP:	yes															
Year	2009															

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) at application
_ underlined values were used for MRL calculation
N/A not applicable

Report:	II A 6.3.2/2 Blaschke U.G. 2001(c) BAS 351 32 H: Determination of the magnitude of the residue of BAS 351 32 H in/on onion raw agricultural commodity specimens from supervised field trials in the Northern Europe in 1999 BASF DocID 2001/1000927
Guidelines:	FAO Guidelines Rome 1990; EEC 96/68; EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8)
GLP:	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

A total number of 2 field trials was conducted in two representative onion growing areas in Northern Europe in 1999. The herbicidal product BAS 351 32 H (480 g/L BAS 351 H, SL) was applied one time at an application rate of 3.2 L/ha corresponding to an application rate of 1.55 kg a.s./ha. The first samples (shallots) were collected 28-30 days after the last application and subsequent samples were taken on day 48-56 (shallots) and 99-100 (onion bulb) days post application. Specimens were analysed by means of BASF Method No. 438/1 (which is a miniaturised version of BASF Method No. 438/0). The limit of quantitation was 0.02 mg/kg for all analysed substances. The study showed that no quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable and the total bentazone residues (expressed as bentazone equivalents) were 0.06 mg/kg on each sampling occasion. Therefore, the residue values were used for MRL determination (see chapter 6.7) even though an exaggerated application rate compared to the critical GAP was used.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** BAS 351 32 H
   Description: Basagran (SL)
   Lot/Batch #: 98-1, 480 g/L BAS 351 H
   Purity: Not reported
   CAS#: 25057-89-0
   Development code: Not reported
   Spiking levels: 0.02 and 0.2 mg/kg (bentazone, 6-OH-bentazone, 8-OH-bentazone)

2. **Test Commodity:**
   Crop: Onion
   Type: Bulb vegetables
   Variety: Renate, Spirit
   Botanical name: *Allium cepa var.*
   Crop parts(s) or processed commodity: Shallots, bulb
   Sample size: 0.5-1 kg (nominal)

B. STUDY DESIGN

1. Test procedure

A total number of 2 supervised field trials was conducted in two representative onion growing areas in Northern France and The Netherlands in 1999. The herbicidal product BAS 351 32 H (480 g/L BAS 351 H, SL) was applied one time at an application rate of 3.2 L/ha corresponding to an application rate of 1.557-1.567 kg a.s./ha. The first samples (shallots) were collected 28-30 days after the last application and subsequent samples were taken on day 48-56 (shallots) and 99-100 (onion bulb) days post application. Collected samples were stored frozen at -20°C until further analysis.

Table 6.3/13 Target application rates and timings for onions

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
1999	2	1	F	BAS 351 32 H (SL)	BAS 351 H	1.552	300	BBCH 13

2. Description of analytical procedures

Residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were determined following BASF Method No. 438/1 (which is a miniaturised version of BASF Method No. 438/0). Bentazone and its metabolites are extracted from plant matrices with aqueous methanol. After purification by an iso-octane liquid-liquid partition, the two relevant metabolites which are present as glucosides are hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic plant constituents, a reversed phase C_{18}-column clean-up is performed. The analytes are then methylated with diazomethane and their derivatives are purified using a silica gel-column. The final determination of the residues of bentazone and its hydroxy metabolites is performed by GC-MS. The limit of quantitation was 0.02 mg/kg for all analysed substances.

II. RESULTS AND DISCUSSION

The residue ranges of bentazone, 6-OH-bentazone and 8-OH-bentazone in onions treated one time with the formulation BAS 351 32 H are shown in Table 6.3/14. Details are presented in Table 6.3/15.
The measured residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in onions treated one time with BAS 351 32 H were below the limit of quantitation of 0.02 mg/kg in each analysed onion sample. As a result, the total residues of bentazone were <0.06 mg/kg on each sampling occasion.
No residues of bentazone or its metabolites were found in the untreated control samples.

Table 6.3/14 Summary of residues in onions

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	1999	28-30	15-41	plant[5]	<0.02	<0.02	<0.02	<0.06
		48-56	19-41	plant[5]	<0.02	<0.02	<0.02	<0.06
		99-100	**49**	**onions**	**<0.02**	**<0.02**	**<0.02**	**<0.06**

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) shallots (bulb and foliage)

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

The study showed that no quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable and the total bentazone residues (expressed as bentazone equivalents) were below 0.06 mg/kg on each sampling occasion.

Table 6.3/15 Residues of bentazone in onion after one application of BAS 351 32 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	BSF 610/002852	Onion	The Netherlands	BAS 351 32 H: 1 x 1.55	13	30	plant[5]	<0.02	<0.02	<0.02	<0.06	**Method:** No. 438/1; LOQ = 0.02 mg/kg				
Doc ID:	2001/1000927					48	plant[5]	<0.02	<0.02	<0.02	<0.06	Bentazone				
Trial No.	BSF/610-1					100	onion	<0.02	<0.02	<0.02	<u>0.06</u>	plant[5]	0.02-0.2	2	104.5	N/A
GLP:	yes											onion bulb	0.02-0.2	2	96.8	N/A
Year	1999											6-OH-bentazone				
Study code:	BSF 610/002852	Onion	France (North)	BAS 351 32 H: 1 x 1.55	13	28	plant[5]	<0.02	<0.02	<0.02	<0.06	plant[5]	0.02-0.2	2	71.0	N/A
Doc ID:	2001/1000927					56	plant[5]	<0.02	<0.02	<0.02	<0.06	onion bulb	0.02-0.2	2	98.4	N/A
Trial No.	BSF/610-2					99	onion	<0.02	<0.02	<0.02	<u>0.06</u>	8-OH-bentazone				
GLP:	yes											plant[5]	0.02-0.2	2	70.4	N/A
Year	1999											onion bulb	0.02-0.2	2	97.8	N/A

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.02 + 0.02 + 0.02 = 0.06; 0.02 + <0.02 + <0.02 = 0.06; <0.02 + <0.02 + <0.02 = <0.06
5) shallots (bulb and foliage)
6) at application
_ underlined values were used for MRL calculation
N/A not applicable

Report:	II A 6.3.2/3 Erdmann H.-P. et al. 2000(b) Study on the residue behavior of Bentazone in onions (ALLSS) after treatment with BAS 351 32 H under field conditions in Germany, 1999 BASF DocID 2000/1018486
Guidelines:	BBA IV 3-3; IVA Guidelines for Residue Studies Sections IA and IB 2nd edition 1992
GLP:	Yes (laboratory certified by Land Brandenburg Ministerium fuer Landwirtschaft, Umweltschutz und Raumordnung, Potsdam, Germany)

Executive Summary

A total number of 2 field trials was conducted in two representative onion growing areas in Northern Europe in 1999. The herbicidal product BAS 351 32 H (480 g/L BAS 351 H, SL) was applied one time at an application rate of 3.2 L/ha corresponding to an application rate of 1.54 kg a.s./ha. The first samples (shallots) were collected 34-39 days after the last application and subsequent samples were taken on day 42-46 (shallots) and 85-96 (onion bulb) days post application. Specimens were analysed by means of BASF Method No. 438/1 (which is a miniaturised version of BASF Method No. 438/0). The limit of quantitation was 0.02 mg/kg for all analysed substances. No quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable and the total bentazone residues (expressed as bentazone equivalents) were below 0.06 mg/kg on each sampling occasion. Therefore, the residue values were used for MRL determination (see chapter 6.7) even though an exaggerated application rate compared to the critical GAP was used.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:** BAS 351 32 H
   Description: Basagran (SL)
   Lot/Batch #: 98-1, 480 g/L BAS 351 H
   Purity: Not reported
   CAS#: 25057-89-0
   Development code: Not reported
   Spiking levels: 0.02 and 0.2 mg/kg (bentazone, 6-OH-bentazone, 8-OH-bentazone)

2. **Test Commodity:**
   Crop: Onion
   Type: Bulb vegetables
   Variety: Stuttgarter Riesen, Zittauer Gelbe
   Botanical name: *Allium cepa*
   Crop parts(s) or processed commodity: Shallots, bulb
   Sample size: Not reported

B. STUDY DESIGN

1. Test procedure

A total number of 2 field trials was conducted in two representative onion growing areas in Germany in 1999. The herbicidal product BAS 351 32 H (480 g/L BAS 351 H, SL) was applied one time at an application rate of 3.16-3.32 L/ha corresponding to an application rate of 1.53-1.61 kg a.s./ha. The first samples (shallots) were collected 34-39 days after the last application and subsequent samples were taken on day 42-46 (shallots) and 85-96 (onion bulb) days post application. Collected samples were stored frozen at -20°C until further analysis.

Table 6.3/16 Target application rates and timings for onions

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
1999	2	1	F	BAS 351 32 H (SL)	BAS 351 H	1.54	300	BBCH 13

2. Description of analytical procedures

Residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were determined following BASF Method No. 438/1 (which is a miniaturised version of BASF Method No. 438/0). Bentazone and its metabolites are extracted from plant matrices with aqueous methanol. After purification by an iso-octane liquid-liquid partition, the two relevant metabolites which are present as glucosides are hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-Bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic plant constituents, a reversed phase C_{18}-column clean-up is performed. The analytes are then methylated with diazomethane and their derivatives are purified using a silica gel-column. The final determination of the residues of bentazone and its hydroxy metabolites is performed by GC/MS. The limit of quantification was 0.02 mg/kg for all analysed substances.

II. RESULTS AND DISCUSSION

The residue ranges of bentazone, 6-OH-bentazone and 8-OH-bentazone in onions treated one time with the single formulation BAS 351 32 H are shown in Table 6.3/17. Details can be found in Table 6.3/18.
The measured residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in onions treated one time with BAS 351 32 H were below the limit of quantitation of <0.02 mg/kg in each analysed onion sample. As a result, the total residues of bentazone (expressed as bentazone equivalents) were below 0.06 mg/kg on each sampling occasion.
No residues of bentazone or its metabolites were found in the untreated control samples.

Table 6.3/17 Summary of residues in onions

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	1999	34-39	18-19	plant[5]	<0.02	<0.02	<0.02	<0.06
		42-46	41	plant[5]	<0.02	<0.02	<0.02	<0.06
		85-96	**49**	**onions**	**<0.02**	**<0.02**	**<0.02**	**<0.06**

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) shallots (bulb and foliage)

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

No quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable and the total bentazone residues (expressed as bentazone equivalents) were 0.06 mg/kg on each sampling occasion.

Table 6.3/18 Residues of bentazone in onion after one application of BAS 351 32 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	AC/BASF/02/99	Onion	Germany	BAS 351 32 H: 1 x 1.54	13	39	plant[5]	<0.02	<0.02	<0.02	<0.06	**Method:** No. 438/1; LOQ = 0.02 mg/kg				
Doc ID:	2000/1018486					46	plant[5]	<0.02	<0.02	<0.02	<0.06	Bentazone				
Trial No.	AC/99/27					96	onion	<0.02	<0.02	<0.02	<u>0.06</u>	plant[5]	0.02-0.2	2	87.4	N/A
GLP:	yes											onion bulb	0.02-0.2	2	86.5	N/A
Year	1999											6-OH-bentazone				
Study code:	AC/BASF/02/99	Onion	Germany	BAS 351 32 H: 1 x 1.54	13	34	plant[5]	<0.02	<0.02	<0.02	<0.06	plant[5]	0.02-0.2	2	81.4	N/A
Doc ID:	2000/1018486					42	plant[5]	<0.02	<0.02	<0.02	<0.06	onion bulb	0.02-0.2	2	70.5	N/A
Trial No.	AC/99/28					85	onion	<0.02	<0.02	<0.02	<u>0.06</u>	8-OH-bentazone				
GLP:	yes											plant[5]	0.02-0.2	2	70.6	N/A
Year	1999											onion bulb	0.02-0.2	2	57.4	N/A

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.02 + 0.02 + 0.02 = 0.06; 0.02 + <0.02 + <0.02 = 0.06; <0.02 + <0.02 + <0.02 = <0.06
5) shallots (bulb and foliage)
6) at application
_ underlined values were used for MRL calculation
N/A not applicable

Spring onion

Report:	II A 6.3.2/4 Schroth E., Martin T. 2010(h) Study on the residue behavior of BAS 351 H (Bentazone) in spring onion after the application of BAS 351 45 H under field conditions in Germany, Netherlands, Greece and Spain, 2009 BASF DocID 2010/1164274
Guidelines:	EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8); EEC 1607/VI/97 rev. 2 10.06.1999; EEC 7029/VI/95 rev. 5; EEC 7525/VI/95 rev. 7
GLP:	Yes (laboratory certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

Executive Summary

During the 2009 growing season, 4 trials in spring onions were conducted in different representative growing areas in Northern and Southern Europe to determine the residue level of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC). BAS 351 45 H (87% bentazone, SG) was applied once at a rate equivalent to 0.722 kg a.s./ha of bentazone in Northern Europe and 0.957 kg a.s./ha in Southern Europe. The application was done at about BBCH 13 in Northern Europe and 30±1 days before harvest in Southern Europe. Specimens of whole plant without roots were collected at the day of the application and at harvest (BBCH 49) in Northern Europe and at the day of application and about 30 days after the application (in case of immaturity an additional sampling of whole plant without roots was done at growth stage BBCH 49) in Southern Europe.
The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. Total bentazone residues spring onion whole plant without roots specimens at harvest were between <0.03-0.06 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 45 H (SG)
Lot/Batch #:	2002-1, 87% bentazone, nominal
Purity:	Not reported
CAS#:	25057-89-0
Development code:	Not reported
Spiking levels:	0.01-10 mg/kg

2. Test Commodity:

Crop:	Onion
Type:	Bulb vegetables
Variety:	Pal, Elody, Degrano
Botanical name:	*Allium*
Crop parts(s) or processed commodity:	- Whole plants without roots
Sample size:	1.0-2.0 kg / 12-24 units

B. STUDY DESIGN

1. Test procedure

During the 2009 growing season, 4 trials in spring onions were conducted in different representative growing areas in Germany, Greece, the Netherlands and Spain to determine the residue level of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).

BAS 351 45 H (87% bentazone, SG) was applied once at a rate equivalent to 0.7221 kg a.s./ha of bentazone in Northern Europe and 0.957 kg a.s./ha in Southern Europe. The spray volume used was 200 L/ha. The application was done at about BBCH 13 in Northern Europe and 30±1 days before harvest in Southern Europe.

Specimens of whole plant without roots were collected at the day of the application and at harvest (BBCH 49) in Northern Europe and at the day of application and about 30 days after the application (in case of immaturity an additional sampling of whole plant without roots was done at growth stage BBCH 49) in Southern Europe. Untreated control specimens were taken at every time point. Specimens were stored frozen (≤-18°C) until analysis. The maximum storage interval from harvest until analysis was 302 days.

Table 6.3/19 Target application rates and timings for spring onions

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2009	2 (N-EU)	1	F	BAS 351 45 H (SG)	BAS 351 H	0.722	200	BBCH 11-14
	2 (S-EU)					0.957		30±1 days before harvest

2. Description of analytical procedures

The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg.

Samples were extracted with aqueous methanol, followed by iso-octane extraction and liquid/liquid partition of an aliquot. Hydrolysis was performed using enzymatic cleavage, and a $Ca(OH)_2$-precipitation step was included to remove acidic matrix constituents. Reversed phase C_{18}-SPE clean-up was performed before the final determination by liquid chromatography and tandem mass spectrometric detection (LC-MS/MS).

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3/20, detailed residue levels are shown in Table 6.3/21 and Table 6.3/22.
Spring onions are a very heterogeneous crop group with a wide range of varieties (with or without bulbs) that were cultivated as annual o perennial crop. Furthermore this crop has no clearly defined physiological ripeness and can be consumed at different developmental stages. This may explain the wide range of BBCH stages at the application (= date of first sampling).
The bentazone residues in spring onion whole plant without roots specimens taken directly after the application ranged between 2.9-11 mg/kg. At 23-24 DALA, residues ranged between <0.01-0.04 mg/kg, and at 30 DALA were below the LOQ (<0.01 mg/kg).
The 6-OH-bentazone residues in spring onion whole plant without roots specimens taken directly after the application ranged between 0.01-0.05 mg/kg. At 23-24 DALA and at 30 DALA residues were below the LOQ (<0.01 mg/kg).
The 8-OH-bentazone residues in spring onion whole plant without roots specimens taken directly after the application ranged between 0.01-0.09 mg/kg. At 23-24 DALA and at 30 DALA residues were below the LOQ (<0.01 mg/kg).
The total bentazone residues in spring onions whole plant without roots specimens taken directly after the application ranged between 2.94-11.13 mg/kg. At harvest the residues declined to levels between 0.03-0.06 mg/kg.
No residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone above the limit of quantitation (0.01 mg/kg) were found in the control specimens of this study.

Table 6.3/20 Summary of residues in spring onions

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	2009	0	13	whole plant[5]	2.9-3.0	0.01-0.02	0.01-0.02	2.94-3.02
		23-24	**49**	**whole plant[5]**	**<0.01-0.04**	**<0.01**	**<0.01**	**<0.03-0.06**
EU South		0	43-45	whole plant[5]	6.2-11	0.02-0.05	0.03-0.09	6.25-11.13
		30	**49**	**whole plant[5]**	**<0.01**	**<0.01**	**<0.01**	**<0.03**

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) without roots

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Total bentazone residues in spring onion whole plant without roots specimens at harvest were between <0.03 and 0.06 mg/kg.

Table 6.3/21 Residues of bentazone in spring onion after one application of BAS 351 45 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	370249	Spring onion	Germany	BAS 351 45 H: 1 x 0.7221	13	0	plant[5]	2.9	0.02	0.02	2.94	**Method:** No. 438/2; LOQ = 0.02 mg/kg				
Doc ID:	2010/1164274					23	plant[5]	0.04	<0.01	<0.01	<u>0.06</u>	Bentazone				
Trial No.	L090378											w. plant[5]	0.01-10	3	98	11.0
GLP:	yes															
Year	2009											6-OH-bentazone				
Study code:	370249	Spring onion	The Netherlands	BAS 351 45 H: 1 x 0.7221	13	0	plant[5]	3.0	0.01	0.01	3.02	w. plant[5]	0.01-0.10	2	98	N/A
Doc ID:	2010/1164274					24	plant[5]	<0.01	<0.01	<0.01	<u><0.03</u>					
Trial No.	L090379											8-OH-bentazone				
GLP:	yes											w. plant[5]	0.01-0.10	2	94	N/A
Year	2009															

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) at application
_ underlined values were used for MRL calculation
N/A not applicable

Table 6.3/22 Residues of bentazone in spring onion after one application of BAS 351 45 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	370249	Spring onion	Spain	BAS 351 45 H: 1 x 0.957	43	0	plant[5]	6.2	0.02	0.03	6.25	**Method:** No. 438/2; LOQ = 0.02 mg/kg				
Doc ID:	2010/1164274					30	plant[5]	<0.01	<0.01	<0.01	<u>≤0.03</u>	Bentazone				
Trial No.	L090380											w. plant[5]	0.01-10	3	98	11.0
GLP:	yes															
Year	2009											6-OH-bentazone				
Study code:	370249	Spring onion	Greece	BAS 351 45 H: 1 x 0.957	45	0	plant[5]	11	0.05	0.09	11.13	w. plant[5]	0.01-0.10	2	98	N/A
Doc ID:	2010/1164274					30	plant[5]	<0.01	<0.01	<0.01	<u>≤0.03</u>					
Trial No.	L090381											8-OH-bentazone				
GLP:	yes											w. plant[5]	0.01-0.10	2	94	N/A
Year	2009															

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) at application
_ underlined values were used for MRL calculation
N/A not applicable

6.3.3 Fruiting vegetables

Sweet corn

Table 6.3/23 GAP for the use of BAS 351 H in/on sweet corn

Crop	Maximum Applied Dose	Water Volume	PHI	Application Method	Application Timing
Sweet corn	1 x 0.96 kg BAS 351 H/ha	200-400 L/ha	28	spray application	12-16

PHI = pre-harvest interval

Table 6.3/24 GAP information of residue trials conducted in sweet corn in 2007

Region	Country	Formulation	Application[0]				DALA[1]
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
EU North	France (1 trial)	BAS 351 40 H, SL	spray application	1.2	0.6	1	0 21 28 35 42
EU South	France (1 trial)	BAS 351 40 H, SL	spray application	1.2	0.6	1	0 22 29 35 42

Report:	II A 6.3.3/1 Oxspring S. 2008(f) Study on the residue behaviour of Bentazone in corn (maize) and sweet corn after treatment with BAS 351 40 H under field conditions in Northern and Southern Europe during 2007 BASF DocID 2008/1049973
Guidelines:	None
GLP:	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report:	II A 6.3.3/2 Oxspring S. 2008(e) Final report amendment number 1: Study on the residue behaviour of Bentazone in corn (maize) and sweet corn after treatment with BAS 351 40 H under field conditions in Northern and Southern Europe during 2007 BASF DocID 2008/1055036
Guidelines:	None
GLP:	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

During the 2007 growing season, 2 field trials were conducted in representative sweet corn growing areas in Northern France and Southern France, to determine the residue level of bentazone (BAS 351 H) and its metabolites BH 351-6-OH and BH 351-8-OH in or on raw agricultural commodities (RAC).
BAS 351 40 H (48% bentazone, SL) was applied once at a rate equivalent to 1.2 kg a.s./ha of bentazone in a spray volume of 200 L/ha. Each trial consisted of a control (untreated) and a treated plot. The application was done 28 days before expected harvest at growth stages BBCH 53-73. Specimens of whole plant without roots were collected at the day of the application (0 DALA). Cobs without husks and rest of plant with husk (no roots) were sampled 21±1, 28±1 and 35 days thereafter. At trial L070786 kernels with cobs and rest of plant with husk (no roots) were sampled at normal commercial harvest (42 DALA) from each plot. At trial L070793, the final commercial harvest specimens were unable to be taken since the grower harvested the trial prior to the planned sampling timing.
The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. At 28±1 PHI (BBCH 79-85), residues between 0.57–0.60 mg/kg and <0.03–0.06 mg/kg were detected in rest of plant without roots and cobs without husks specimens, respectively. At expected harvest of 42 days after the last application (BBCH 87), total bentazone residues in kernels with cobs and rest of plant (no roots) specimens were <0.03 and 0.36 mg/kg, respectively.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 40 H (SL)
Lot/Batch #:	12664, 48% bentazone, nominal
Purity:	Not reported
CAS#:	25057-89-0
Development code:	
Spiking levels:	0.01-20 mg/kg

2. Test Commodity:

Crop:	Sweet corn
Type:	Fruiting vegetables
Variety:	Challenger, GH 5704
Botanical name:	*Zea mays* L., var. *saccharata*
Crop parts(s) or processed commodity:	Whole plant, cobs without husk, rest of plant (no roots), grain
Sample size:	1.0 kg (12 units)

B. STUDY DESIGN

1. Test procedure

During the 2007 growing season, 2 field trials were conducted in representative sweet corn growing areas in Northern France and Southern France, to determine the residue level of bentazone (BAS 351 H) and its metabolites BH 351-6-OH and BH 351-8-OH in or on raw agricultural commodities (RAC).

BAS 351 40 H (48% bentazone, SL) was applied once at a rate equivalent to 1.2 kg a.s./ha of bentazone in a spray volume of 200 L/ha. Each trial consisted of a control (untreated) and a treated plot. The application was done 28 days before expected harvest at growth stages BBCH 53-73. Specimens of whole plant without roots were collected at the day of the application (0 DALA). Cobs without husks and rest of plant with husk (no roots) specimens were sampled 21±1, 28±1 and 35 days thereafter. At trial L070786 kernels with cobs and rest of plant with husk (no roots) were sampled at normal commercial harvest (42 DALA) from each plot. At trial L070793, the final commercial harvest specimens were unable to be taken since the grower harvested the trial prior to the planned sampling timing.

Untreated control specimens were taken at every time point. Specimens were stored frozen (≤-18°C) until analysis. The maximum storage interval from harvest until analysis was 324 days.

Table 6.3/25Target application and timings for sweet corn

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2007	2	1	F	BAS 351 40 H (SL)	BAS 351 H	1.2	200	28±1 days before harvest

2. Description of analytical procedures

The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg.
Samples were extracted with aqueous methanol, followed by iso-octane extraction and liquid/liquid partition of an aliquot. Hydrolysis was performed using enzymatic cleavage, and a $Ca(OH)_2$-precipitation step was included to remove acidic matrix constituents. Reversed phase C_{18}-SPE clean-up was performed before the final determination by liquid chromatography and tandem mass spectrometric detection (HPLC-MS/MS).

II. RESULTS AND DISCUSSION

The summary of residues in sweet corn is shown in Table 6.3/26, detailed residue levels are shown in Table 6.3/27 and Table 6.3/28.
Directly after application (0 DALA, BBCH 53-73), residues of BAS 351 H and its metabolites ranged from 5.43 to 10.31 mg/kg in whole plants (without roots). BAS 351 H accounted for 3.51 to 7.75 mg/kg of the total residue, while 6-OH-bentazone (BH 351-6-OH) and 8-OH-bentazone (BH 351-8-OH) was detected with 1.86 to 2.20 mg/kg and 0.19 to 0.53 mg/kg, respectively.
At 21±1 days after application residue levels had decreased significantly. For BAS 351 H and its metabolite BH 351-8-OH no residues above the limit of quantitation were detected in cobs without husk. BH 351-6-OH was present in cobs without husk at levels of between <0.01 and 0.02 mg/kg. Residue levels in rest of plant (without roots) samples also decreased to values of <0.01 to 0.10 mg/kg for BAS 351 H and 0.33 to 0.54 mg/kg for BH 351-6-OH. The metabolite BH 351-8-OH was detected in rest of plant (without roots) samples at 21±1 days after application at levels of 0.01 to 0.02 mg/kg.
At 28±1 DALA residue levels of BH 351-6-OH of between <0.01 and 0.04 mg/kg were detected in cobs without husk; whilst no residues of BAS 351 H or BH 351-8-OH were detected above the limit of quantitation in the same specimens. Residue levels detected in rest of plant (without roots) samples were between 0.01 and 0.09 mg/kg for BAS 351 H, 0.53 and 0.56 mg/kg for BH 351-6-OH and <0.01 and 0.03 mg/kg for BH 351-8-OH.
At 35±1 DALA no residues above the limit of quantitation were detected in cobs without husk for BAS 351 H, BH 351-6-OH or BH 351 8-OH. Residues detected in the rest of plant (without roots) were 0.49 mg/kg and 0.02 mg/kg for BH 351-6-OH and BH 351-8-OH, respectively, while no residues above the limit of quantitation were found for BAS 351 H.

At maturity (BBCH 87, 42 DALA) no residues above the limit of quantitation were detected in kernels with cobs for BAS 351 H, BH 351-6-OH or BH 351 8-OH. Residues detected in the rest of plant (without roots) were 0.35 mg/kg and 0.02 mg/kg for BH 351-6-OH and BH 351-8-OH, respectively, while no residues above the limit of quantitation were found for BAS 351 H.

No residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone above the limit of quantitation (0.01 mg/kg) were found in the control specimens of this study.

Table 6.3/26 Summary of residues in sweet corn

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU South + EU North	2007	0	53-73	whole plant[5]	3.51-7.75	1.86-2.20	0.19-0.53	5.43-10.31
		21-22	65-79	cobs w/o husk	<0.01	<0.01-0.02	<0.01	<0.03-0.04
		28-29	**79-85**	**cobs w/o husk**	**<0.01**	**<0.01-0.04**	**<0.01**	**<0.03-0.06**
		35	85	cobs w/o husk	<0.01	<0.01	<0.01	<0.03
		21-22	65-79	rest of plant[6)]	<0.01-0.10	0.33-0.54	0.01-0.02	0.33-0.63
		28-29	79-85	rest of plant[6)]	0.01-0.09	0.53-0.56	<0.01-0.03	0.57-0.60
		35	85	rest of plant[6)]	<0.01	0.49	0.02	0.49
		42	87	rest of plant[6)]	<0.01	0.35	0.02	0.36
		42	87	kernels w. cobs	<0.01	<0.01	<0.01	<0.03

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) without roots
6) with husk (w/o roots)

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

At 28±1 DALA residue levels of 6-OH-bentazone (BH 351-6-OH) of between <0.01 and 0.04 mg/kg were detected in cobs without husk; no residues of bentazone (BAS 351 H) or 8-OH-bentazone (BH 351-8-OH) were detected above the limit of quantitation in the same specimens. Total bentazone residues ranged from <0.03-0.06 mg/kg.

Table 6.3/27 Residues of bentazone in sweet corn after one application of BAS 351 40 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Benta-zone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	303258	Sweet corn	France	BAS 351 40 H: 1 x 1.2	53-55	0	whole plant[5)]	7.75	2.20	0.53	10.31	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2008/1055036					21	cobs w/o husk	<0.01	<0.01	<0.01	<0.03	Bentazone				
	2008/1049973					21	rest of plant[7)]	<0.01	0.33	0.01	0.33	cob / grain	0.01-1.0	9	88	15
Trial No.	L070786					28	cobs w/o husk	<0.01	<0.01	<0.01	<u>0.03</u>	rest of pl.	0.01-20.0	26	91	10
GLP:	yes					28	rest of plant[7)]	0.01	0.56	0.03	0.57	6-OH-bentazone				
Year	2007					35	cobs w/o husk	<0.01	<0.01	<0.01	<0.03	cob / grain	0.01-1.0	12	90	11
						35	rest of plant[7)]	<0.01	0.49	0.02	0.49	rest of pl.	0.01-20.0	25	83	8
						42	kernels w. cobs	<0.01	<0.01	<0.01	<0.03	8-OH-bentazone				
						42	rest of plant[7)]	<0.01	0.35	0.02	0.36	cob / grain	0.01-1.0	8	83	8
												rest of pl.	0.01-20.0	28	88	12

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) at application
7) with husk (no roots)
_ underlined values were used for MRL calculation

Table 6.3/28 Residues of bentazone in sweet corn after one application of BAS 351 40 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Benta-zone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	303258	Sweet corn	France	BAS 351 40 H: 1 x 1.2	73	0	whole plant[5)]	3.51	1.86	0.19	5.43	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2008/1055036					22	cobs w/o husk	<0.01	0.02	<0.01	0.04	Bentazone				
	2008/1049973					22	rest of plant[7)]	0.10	0.54	0.02	0.63	cob / grain	0.01-1.0	9	88	15
Trial No.	L070793					29	cobs w/o husk	<0.01	0.04	<0.01	<u>0.06</u>	rest of pl.	0.01-20.0	26	91	10
GLP:	yes					29	rest of plant[7)]	0.09	0.53	<0.01	0.60	6-OH-bentazone				
Year	2007											cob / grain	0.01-1.0	12	90	11
												rest of pl.	0.01-20.0	25	83	8
												8-OH-bentazone				
												cob / grain	0.01-1.0	8	83	8
												rest of pl.	0.01-20.0	28	88	12

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) at application
7) with husk (no roots)
_ underlined values were used for MRL calculation

6.3.4 Fresh herbs

Thyme

Table 6.3/29 Representative GAP for the use of BAS 351 H in/on thyme

Crop	Maximum Applied Dose	Water Volume	PHI	Application Method	Application Timing
Chive Mint	1 x 0.96 kg BAS 351 H/ha	200-400 L/ha	F	spray application	11-13

PHI = pre-harvest interval

The critical GAP for basil, chive and mint is presented here as these crops belong to the same crop group as thyme. Since for thyme no critical GAP has been established yet, the respective trials are compared to the cGAP for basil, chive and mint.

Table 6.3/30 GAP information of residue trials conducted in thyme in 2005-2006

Crop	Region	Country	Formulation	Application[0]				DALA[1]
				Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
Thyme	EU South	France (4 trials)	Basagran (BAS 351 45 H) (870 g a.s./L, SG)	spray application	1.0-1.1	0.21-0.26	1	28

0) actual application rates varied by 10% at most
1) days after last application

Report: II A 6.3.4/1
Malet J.C., Allard L. 2007(e)
Mesure du niveau de residu de Bentazone, apres 1 application de la preparation BASAGRAN SG sur thym dans le cadre d une extension d usage sur la culture
BASF DocID 2007/1063041

Guidelines: EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8); EEC 7029/VI/95 rev. 5

GLP: Yes
(laboratory certified by Groupe Interministeriel des Produits Chimiques, France)

Note: An English translation of the report is provided in DocID 2011/1072551

Executive Summary

During the 2005 growing season 2 field trials were conducted in thyme in Southern France to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
Therefore the formulated product Basagran (870 g/L bentazone, SG) was applied once at a rate equivalent to 1.1 kg a.s./ha in a spray volume of approximately 500 L/ha. Each trial consisted of a control (untreated) and a treated plot. The application was done at 28 days before the expected harvest at growth stages BBCH 72-73. Specimens of whole plant were collected 28 days after the last application (28 DALA).
The thyme specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone using GC-MS/MS analytical method, which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.05 mg/kg for all analytes, expressed as sum of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone (0.02 mg/kg + 2x 0.014 mg/kg).
The total bentazone residue level in the treated thyme specimens 28 days after the last application (28 DALA) were <0.033-0.072 mg/kg. No residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone above the limit of quantitation (0.05 mg/kg) were found in the control specimens of this study.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 45 H, Basagran (SG)
Lot/Batch #:	97-4153, 870 g/L bentazone, nominal
Purity:	
CAS#:	25057-89-0 (bentazone)
Development code:	
Spiking levels:	0.014-0.02 mg/kg

2. Test Commodity:

Crop:	Thyme
Type:	Fresh herbs
Variety:	Population, Volt 2001 ameliore
Botanical name:	*Thymus vulgaris* L.
Crop parts(s) or processed commodity:	Whole plant
Sample size:	Approximately 1.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2005 growing season, 2 field trials were conducted with thyme in Southern France, to determine the residue level of bentazone (BAS 351 H) and its metabolites BH 351-6-OH (6-OH-bentazone) and BH 351-8-OH (8-OH-bentazone) in or on raw agricultural commodities (RAC).
Therefore 1.2 kg formulated product/ha Basagran (87% bentazone, SG) was applied once at a rate equivalent to 1.04 kg a.s./ha in a spray volume of approximately 500 L/ha. Each trial consisted of a control (untreated) and a treated plot. The application was done at 28 days before expected harvest at growth stages BBCH 72-73. Specimens of whole plant were collected 28 days after the last application (28 DALA).

Table 6.3/31 Target application and timings for thyme

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2005	2	1	F	Basagran (SG)	BAS 351 H	1.0	500	28±1 days before harvest

2. Description of analytical procedures

The thyme specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone using GC-MS/MS analytical method, which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.05 mg/kg for all analytes, expressed as sum of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone.
Prior to the final determination, the residues of bentazone and its metabolites were extracted from thyme specimens using a methanol/water mixture.

II. RESULTS AND DISCUSSION

The summary of residues in thyme is shown in Table 6.3/32, detailed residue levels are shown in Table 6.3/33.
The residues of bentazone in the treated thyme specimens 28 days after the last application (28 DALA) were <0.02-0.037 mg/kg, those of 6-OH-bentazone and 8-OH-bentazone between <0.014 and 0.023 mg/kg, respectively. In thyme specimens, total bentazone was <0.05-0.07 mg/kg.
No residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone above the limit of quantitation (0.05 mg/kg) were found in the control specimens of this study.

Table 6.3/32 Summary of residues in thyme

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Benta-zone[3]	8-OH-Benta-zone[4]	Total Benta-zone[5]
EU South	2005	**28**	**72-73**	**whole plant**	**<0.02-0.037**	**<0.014-0.023**	**<0.014**	**<0.05-0.07**

1) days after last application
2) growth stage at application
3) expressed as 6-OH-bentazone
4) expressed as 8-OH-bentazone
5) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938

LOQ = 0.05 mg/kg for total bentazone expressed as sum of 0.02 mg/kg (bentazone) + 0.014 mg/kg (6-OH-bentazone) + 0.014 mg/kg (8-OH-bentanzone)

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

The total bentazone residues in thyme whole plant specimens determined after one application of Basagran (87% bentazone, SG) were <0.05-0.07 mg/kg 28 days after the last application (28 DALA).

Table 6.3/33 Residues of bentazone in thyme after one application of Basagran (87% bentazone, SG) in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Benta-zone	6-OH-Benta-zone[3]	8-OH-Benta-zone[4]	Total Benta-zone[5]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	RLTH29505	Thyme	France	Basagran: 1 x 1.1	72	28	whole plant	0.037	0.023	<0.014	0.072	GC-MS/MS (GIRPA laboratory); LOQ = 0.02 mg/kg (bentazone), 0.014 mg/kg (6-OH-, 8-OH-)				
Doc ID:	2007/1063041											Bentazone				
Trial No.	RE05073											whole plant	0.02	3	82	15
GLP:	yes															
Year	2005											6-OH-bentazone				
Study code:	RLTH29505	Thyme	France	Basagran: 1 x 1.1	73	28	whole plant	<0.02	<0.014	<0.014	<0.05	whole plant	0.014	3	80	10
Doc ID:	2007/1063041															
Trial No.	RE05074											8-OH-bentazone				
GLP:	yes											whole plant	0.014	3	103	14
Year	2005															

0) actual application rates varied by 10% at most
1) days after last application
2) growth stage at application
3) expressed as 6-OH-bentazone
4) expressed as 8-OH-bentazone
5) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
n.r. not reported
_ underlined values were used for MRL calculation

Report:	II A 6.3.4/2 Malet J.C., Allard L. 2007(f) Mesure du niveau de residu de Bentazone, apres 1 application de la preparation BASAGRAN SG sur thym dans le cadre d une extension d usage sur la culture BASF DocID 2008/1065207
Guidelines:	EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8); EEC 7029/VI/95 rev. 5
GLP:	Yes (laboratory certified by Groupe Interministeriel des Produits Chimiques, France)

Note: An English translation of the report is provided in DocID 2011/1072550

Executive Summary

During the 2006 growing season, 2 field trials were conducted in thyme in Southern France to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
Therefore 1.2 kg formulated product/ha Basagran (87% bentazone, SG) was applied once at a rate equivalent to 1.0-1.1 kg a.s./ha of bentazone in a spray volume of 400 L/ha. Each trial consisted of a control (untreated) and a treated plot. The application was done at 28 days before expected harvest at growth stage BBCH 33. Specimens of whole plant were collected 28 days after the last application (28 DALA).
The thyme specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone using GC-MS/MS analytical method, which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.05 mg/kg for all analytes, expressed as total residue.
The total bentazone residue level in the treated thyme specimens 28 days after the last application (28 DALA) were 0.061-0.087 mg/kg. No residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone above the limit of quantitation (LOQ) were found in the control specimens of this study.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 45 H, Basagran (SG)
Lot/Batch #:	2002-1, Bentazone: 870 g/L, nominal
Purity:	
CAS#:	25057-89-0 (bentazone)
Development code:	
Spiking levels:	0.014-0.02 mg/kg

2. Test Commodity:

Crop:	Thyme
Type:	Fresh herbs
Variety:	Population, Varico
Botanical name:	*Thymus vulgaris* L.
Crop parts(s) or processed commodity:	Whole plant
Sample size:	Approximately 1.0 kg (12 units)

B. STUDY DESIGN

1. Test procedure

During the 2006 growing season 2 field trials were conducted with thyme in Southern France to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
Therefore 1.1-1.2 kg formulated product/ha Basagran (870 g/L bentazone, SG) was applied once at a rate equivalent to 1.0-1.1 kg a.s./ha of bentazone in a spray volume of 400 L/ha. Each trial consisted of a control (untreated) and a treated plot. The application was done at 28 days before expected harvest at growth stage BBCH 33. Specimens of whole plant were collected 28 days after the last application (28 DALA).

Table 6.3/34 Target application and timings for thyme

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2006	2	1	F	BASAGRAN (SG)	BAS 351 H	1.0	400	BBCH 33

2. Description of analytical procedures

The thyme specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone using GC-MS/MS analytical method, which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.05 mg/kg for all analytes, expressed as sum of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone.

Prior to the final determination, the residues of bentazone and its metabolites were extracted from thyme specimens using a methanol/water mixture.

II. RESULTS AND DISCUSSION

The summary of residues in thyme is shown in Table 6.3/35, detailed residue levels are shown in Table 6.3/36. In this study only total bentazone residues, expressed as sum of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone were presented.

The total bentazone residues in the treated thyme specimens 28 days after the last application (28 DALA) were 0.061-0.087 mg/kg. No residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone above the limit of quantitation (0.05 mg/kg) were found in the control specimens of this study.

Table 6.3/35 Summary of residues in thyme

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone	8-OH-Bentazone	Total Bentazone[3]
EU South	2006	**28**	**33**	**whole plant**	**n.r.**	**n.r.**	**n.r.**	**0.061-0.087**

1) days after last application
2) growth stage at application
4) total bentazone expressed as sum of parent bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone, the conversion factor for both metabolites is 0.938

n.r. not reported
Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

The total bentazone residues in thyme whole plant specimens determined after one application of Basagran (870 g/L bentazone, SG) were 0.061-0.087 mg/kg 28 days after the last application (28 DALA).

Table 6.3/36 Residues of bentazone in thyme after one application of Basagran (87% bentazone, SG) in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Benta-zone	6-OH-Benta-zone[3]	8-OH-Benta-zone[4]	Total Benta-zone[5]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	RLTH25306	Thyme	France	Basagran: 1 x 1.1	33	28	whole plant	n.r.	n.r.	n.r.	0.061	GC-MS/MS (GIRPA laboratory); LOQ = 0.05 mg/kg (bentazone, 6-OH-, 8-OH-)				
Doc ID:	2008/1065207											Bentazone, 6-OH-bentazone, 8-OH-bentazone				
Trial No.	RE06093											whole plant	0.05	n.r.	70	n.r.
GLP:	yes															
Year	2006															
Study code:	RLTH25306	Thyme	France	Basagran: 1 x 1.0	33	28	whole plant	n.r.	n.r.	n.r.	0.087					
Doc ID:	2008/1065207															
Trial No.	RE06094															
GLP:	yes															
Year	2006															

0) actual application rates varied by 10% at most
1) days after last application
2) growth stage at application
3) expressed as 6-OH-bentazone
4) expressed as 8-OH-bentazone
5) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
n.r. not reported
_ underlined values were used for MRL calculation

6.3.5 Legume vegetables

Table 6.3/37 GAP for the use of BAS 351 H in/on legume vegetables

Crop	Maximum Applied Dose	Water Volume	PHI	Application Method	Application Timing
Bean with pod Bean without pod	1 x 0.96 kg BAS 351 H/ha	200-400 L/ha	28	spray application	BBCH 10-15
Pea with pod Pea without pod	1 x 0.96 kg BAS 351 H/ha	200-400 L/ha	28	spray application	BBCH 11-15

PHI = pre-harvest interval

Table 6.3/38 GAP information of residue trials conducted in green beans between 2008 and 2009

Region	Country	Formulation	Application[0]				DALA[1]
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
EU North	Germany (2 trials)	BAS 351 45 H, SG	spray application	1.200	0.600	1	0 28-29 34-36 41-43
	The Netherlands (1 trial)						
	United Kingdom (2 trials)	BAS 351 45 H, SG	spray application	0.957	0.479	1	0 27-29 35 41-42
	France (2 trials)						
EU South	France (1 trial)	BAS 351 45 H, SG	spray application	1.200	0.600	1	0 28-29 35-36 42
	Italy (1 trial)						
	Spain (2 trials)						
	Greece (1 trial)						
	France (2 trials)	BAS 351 45 H, SG	spray application	0.957	0.479	1	0 27-28 35-36 40-42
	Spain (2 trials)						

0) actual application rates varied by 10% at most
1) days after last application

Table 6.3/39 GAP information of residue trials conducted in green peas between 1999 and 2007

Region	Country	Formulation	Application[0]				DALA[1]
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
EU North	United Kingdom (2 trials)	BAS 351 45 H, SG	spray application	0.957	0.479	1	0 28
	United Kingdom (4 trials)	BAS 351 45 H, SG	spray application	1.440	0.480	1	0 38-51
	United Kingdom (4 trials)	BAS 351 53 H, SG	spray application	1.440	0.480	1	0 38-51

0) actual application rates varied by 10% at most
1) days after last application

Beans

Report: II A 6.3.5/1
Schroth E., Martin T. 2010(i)
Study on the residue behavior of BAS 351 H (Bentazone) in bean after the application of BAS 351 45 H under field conditions in Germany, Netherlands, Greece, France (South), Italy and Spain, 2009
BASF DocID 2009/1123296

Guidelines: EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8); EEC 1607/VI/97 rev. 2 10.06.1999; EEC 7029/VI/95 rev. 5; EEC 7525/VI/95 rev. 7

GLP: Yes
(laboratory certified by ENAC, Entidad Nacional de Acreditación, Madrid, Spain)

Executive Summary

During the 2009 growing season, 8 trials in green beans were conducted in different representative growing areas in Northern and Southern Europe to determine the residue level of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC). BAS 351 45 H (87% bentazone, SG) was applied once at a rate equivalent to 1.2 kg a.s./ha of bentazone. The application was done at crop growth stage BBCH 55. Specimens of whole plant without roots were collected at the day of the application. Specimens of rest of plant without roots, pods with seeds, pods without seeds and seeds were collected about 28 and 35 days after the application. Specimens of rest of plant without roots, pods without seeds and seeds were taken about 42 days after the application.
The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. Total bentazone residues were <0.03-0.21 mg/kg in pods with seeds and <0.03-0.04 mg/kg in seeds at 28-29 DALA. Specimens taken at 34-36 DALA did not show major differences (pods with seeds <0.03-0.10 mg/kg and seeds <0.03-0.03 mg/kg). At 41-43 DALA <0.03-0.40 mg/kg in pods without seeds and <0.03-0.04 mg/kg in seeds were found.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 45 H (SG)
Lot/Batch #:	2002-1, 87% bentazone, nominal
Purity:	Not reported
CAS#:	25057-89-0
Development code:	Not reported
Spiking levels:	0.01-200 mg/kg

2. Test Commodity:

Crop:	Green bean
Type:	Legume vegetables
Variety:	Kansas, Nassau, Artemis, Linex, Flavert, Tremaya, Etna
Botanical name:	*Phaseolus vulgaris*
Crop parts(s) or processed commodity:	Whole plants without roots, pods with seeds, pods without seeds, seeds, rest of plant without root
Sample size:	Min. 0.2-1.0 kg / 12-24 units

B. STUDY DESIGN

1. Test procedure

During the 2009 growing season, 8 trials in green beans were conducted in different representative growing areas in Germany, the Netherlands, Greece, Southern France, Italy and Spain to determine the residue level of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
BAS 351 45 H (87% bentazone, SG) was applied once at a rate equivalent to 1.2 kg a.s./ha of bentazone and the spray volume used was 200 L/ha. The application was done at crop growth stage BBCH 55.
Specimens of whole plant without roots were collected at the day of the application. Specimens of rest of plant without roots, pods with seeds, pods without seeds and seeds were collected about 28 and 35 days after the application. Specimens of rest of plant without roots, pods without seeds and seeds were taken about 42 days after the application. Untreated control specimens were taken at every time point. Specimens were stored frozen (≤-18°C) until analysis. The maximum storage interval from harvest until analysis was 228 days.

Table 6.3/40 Target application rates and timings for green beans

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2009	8	1	F	BAS 351 45 H (SG)	BAS 351 H	1.2	200	BBCH 55

2. Description of analytical procedures

The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg.
Bentazone and its metabolites are extracted from plant or animal matrices with aqueous methanol. After purification of an aliquot by an iso-octane liquid-liquid partition, the two relevant metabolites which are present as glucosides are hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic matrix constituents, a reversed phase C_{18}-SPE clean-up is performed. The final determination of bentazone and its hydroxyl-metabolites is performed by LC-MS/MS, monitoring two parent-daughter ion transitions for each compound.

II. RESULTS AND DISCUSSION

A summary of residues is given in Table 6.3/41. Details can be found in Table 6.3/42 and Table 6.3/43.

Directly after application bentazone residues in whole plant without roots were found in the range of 37.57 to 131.89 mg/kg. These residues degraded rapidly during the course of the study to <0.01-0.02 mg/kg in pods with seeds and <0.01 mg/kg in pods without seeds and seeds and <0.01-0.06 mg/kg in rest of plant without roots at 28-29 DALA. Specimens taken at 34-36 DALA did not show major differences (pods without seeds and seeds remained at <0.01 mg/kg, whereas pods with seeds (<0.01 mg/kg) and rest of plant without roots (<0.01-0.02 mg/kg) showed a slight decrease). At 41-43 DALA the residues remained at the same level (pods without seeds <0.01 mg/kg, seeds and rest of plant without roots <0.01-0.02 mg/kg).

No residues of bentazone above the limit of quantitation were found in any of the analysed untreated specimens.

Directly after application 6-OH-bentazone residues in whole plant without roots were found in the range of 0.35 to 0.95 mg/kg. These residues degraded rapidly during the course of the study to <0.01-0.18 mg/kg in pods with seeds, 0.03-0.20 mg/kg in pods without seeds and <0.01-0.02 mg/kg in seeds and increased to 1.21-8.61 mg/kg in rest of plant without roots at 28-29 DALA. Specimens taken at 34-36 DALA did not show major differences (pods with seeds <0.01-0.09 mg/kg, pods without seeds 0.01-0.21 mg/kg and seeds <0.01-0.01 mg/kg) apart from the rest of plant without roots specimens which showed a slight decrease to 0.38-5.73 mg/kg. At 41-43 DALA residues remained at the same level (pods without seeds <0.01-0.31 mg/kg, seeds <0.01 mg/kg and rest of plant without roots 0.20-6.72 mg/kg).

No residues of 6-OH-bentazone above the limit of quantitation were found in any of the analysed untreated specimens apart from two specimens from the Greek trial (L090403) where 0.07 mg/kg were determined in pods without seeds at 35 DALA and 0.11 mg/kg in rest of plant without roots at 35 DALA.

Directly after application 8-OH-bentazone residues in whole plant without roots were found in the range of 0.27 to 0.68 mg/kg. These residues degraded rapidly during the course of the study to <0.01-0.03 mg/kg in pods with seeds, 0.01-0.06 mg/kg in pods without seeds and <0.01 mg/kg in seeds specimens and remained at the same level in rest of plant without roots (0.12-0.66 mg/kg) at 28-29 DALA. Specimens taken at 34-36 DALA did not show major differences (pods with seeds <0.01-0.03 mg/kg, pods without seeds <0.01-0.06 mg/kg, seeds <0.01 mg/kg and rest of plant without roots 0.06-0.79 mg/kg). At 41-43 DALA <0.01-0.10 mg/kg in pods without seeds, <0.01 mg/kg in seeds and 0.02-0.77 mg/kg in rest of plant without roots were found.

No residues of 8-OH-bentazone above the limit of quantitation were found in any of the analysed untreated specimens apart from one specimen from the Greek trial (L090403) where 0.03 mg/kg were determined in pods without seeds at 35 DALA.

Directly after application total bentazone residues in whole plant without roots were found in the range of 38.15 to 133.01 mg/kg. These residues degraded rapidly during the course of the study to <0.03-0.21 mg/kg in pods with seeds, 0.05-0.25 mg/kg in pods without seeds, <0.03-0.04 mg/kg in seeds and 1.34-8.68 mg/kg in rest of plant without roots at 28-29 DALA. Specimens taken at 34-36 DALA did not show major differences (pods with seeds <0.03-0.10 mg/kg, pods without seeds 0.03-0.26 mg/kg and seeds <0.03-0.03 mg/kg) apart from the rest of plant without roots which showed a slight decrease to 0.42-6.14 mg/kg. At 41-43 DALA <0.03-0.40 mg/kg in pods without seeds, <0.03-0.04 mg/kg in seeds and 0.22-7.05 mg/kg in rest of plant without roots were found.

No residues of total bentazone above the limit of quantitation were found in any of the analysed untreated specimens apart from two specimens from the Greek trial (L090403) where 0.09 mg/kg were determined in pods without seeds at 35 DALA and 0.11 mg/kg in rest of plant without roots at 35 DALA.

Table 6.3/41 Summary of residues in green beans

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	2009	0	55	whole plant[5]	37.57-71.64	0.35-0.61	0.27-0.68	38.15-72.86
		28-29	**81-83**	**pods***	**<0.01-0.02**	**<0.01-0.07**	**<0.01**	**<0.03-0.09**
		34-36	85-87	pods*	<0.01	0.02-0.09	<0.01-0.01	0.04-0.10
		28-29	81-83	rest plant[5]	<0.01-0.02	1.65-5.25	0.22-0.61	1.77-5.53
		34-36	85-87	rest plant[5]	<0.01-0.01	1.94-5.73	0.24-0.79	2.06-6.14
		41-43	89	rest plant[5]	<0.01-0.01	1.21-6.72	0.19-0.77	1.33-7.05
		28	83	pods**	<0.01	0.07	0.02	0.09
		34-36	85-87	pods**	<0.01	0.03-0.09	0.01-0.03	0.05-0.12
		41-43	89	pods**	<0.01	0.04-0.05	0.01-0.02	0.06-0.08
		28	**83**	**seed**	**<0.01**	**<0.01**	**<0.01**	**<0.03**
		34-36	85-87	seed	<0.01	<0.01-0.01	<0.01	<0.03-0.03
		41-43	89	seed	<0.01	<0.01	<0.01	<0.03
EU South	2009	0	55	whole plant[5]	77.50-131.89	0.45-0.95	0.30-0.63	78.65-133.01
		28-29	**75-79**	**pods***	**<0.01**	**0.01-0.18**	**<0.01-0.03**	**0.03-0.21**
		35-36	73-85	pods*	<0.01	<0.01-0.07	<0.01-0.03	<0.03-0.10
		28-29	69-79	rest plant[5]	0.01-0.06	1.21-8.61	0.12-0.66	1.34-8.68
		35-36	73-85	rest plant[5]	<0.01-0.02	0.38-4.08	0.06-0.52	0.42-4.33
		42	79-97	rest plant[5]	<0.01-0.02	0.20-3.47	0.02-0.43	0.22-3.64
		28-29	75-79	pods**	<0.01	0.03-0.20	0.01-0.06	0.05-0.25
		35	77-85	pods**	<0.01	0.01-0.21	0.01-0.06	0.03-0.26
		42	79-97	pods**	<0.01	<0.01-0.31	<0.01-0.10	<0.03-0.40
		28-29	**75-79**	**seed**	**<0.01**	**<0.01-0.02**	**<0.01**	**<0.03-0.04**
		35	77-85	seed	<0.01	<0.01	<0.01	<0.03
		42	79-97	seed	<0.01-0.02	<0.01	<0.01	<0.03-0.04

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) without roots
* with seeds
** without seeds
Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Total bentazone residues were <0.03-0.21 mg/kg in pods with seeds and <0.03-0.04 mg/kg in seeds at 28-29 DALA. Specimens taken at 34-36 DALA did not show major differences (pods with seeds <0.03-0.10 mg/kg and seeds <0.03-0.03 mg/kg). At 41-43 DALA <0.03-0.40 mg/kg in pods without seeds and <0.03-0.04 mg/kg in seeds were found.

Table 6.3/42 Residues of bentazone in green beans after one application of BAS 351 45 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	370250	Green bean	Germany	BAS 351 45 H: 1 x 1.2	55	0	plant[5]	67.89	0.48	0.40	68.72	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2009/1123296					29	pods*	0.02	0.02	<0.01	0.05	Bentazone				
Trial No.	L090396					29	rest pl. [5]	0.02	1.94	0.29	2.11	w. plant[5]	0.01-200	7	99	16
GLP:	yes					36	pods*	<0.01	0.04	<0.01	0.06	pods*	0.01-0.10	4	96	7.9
Year	2009					36	pods**	<0.01	0.03	0.01	0.05	pods**	0.01-2.0	8	107	8.2
						36	seed	<0.01	<0.01	<0.01	<0.03	seed	0.01-0.10	6	106	7.1
						36	rest pl. [5]	0.01	5.73	0.79	6.14	rest pl. [5]	0.01-10	7	106	11
						43	pods**	<0.01	0.04	0.01	0.06	6-OH-bentazone				
						43	seed	<0.01	<0.01	<0.01	<0.03	w. plant[5]	0.01-5.0	6[#]	93	15
						43	rest pl. [5]	<0.01	1.21	0.19	1.33	pods*	0.01-0.20	4	105	10
Study code:	370250	Green bean	Germany	BAS 351 45 H: 1 x 1.2	55	0	plant[5]	71.64	0.61	0.68	72.86	pods**	0.01-1.0	6	108	4.4
Doc ID:	2009/1123296					29	pods*	<0.01	<0.01	<0.01	<0.03	seed	0.01-0.10	6	103	10
Trial No.	L090397					29	rest pl. [5]	<0.01	1.65	0.22	1.77	rest pl. [5]	0.01-10	6	107	14
GLP:	yes					35	pods*	<0.01	0.02	<0.01	0.04	8-OH-bentazone				
Year	2009					35	pods**	<0.01	0.03	0.01	0.05	w. plant[5]	0.01-5.0	7	109	20
						35	seed	<0.01	0.01	<0.01	0.03	pods*	0.01-0.20	4	85	7.6
						35	rest pl. [5]	<0.01	1.94	0.24	2.06	pods**	0.01-1.0	6	102	12
						43	pods**	<0.01	0.04	0.02	0.07	seed	0.01-0.10	6	92	15
						43	seed	<0.01	<0.01	<0.01	<0.03	rest pl. [5]	0.01-10	6	104	11
						43	rest pl. [5]	0.01	1.86	0.27	2.02					

Table 6.3/42 Residues of bentazone in green beans after one application of BAS 351 45 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	370250	Green bean	The Netherlands	BAS 351 45 H: 1 x 1.2	55	0	plant[5]	37.57	0.35	0.27	38.15					
Doc ID:	2009/1123296					28	pods*	<0.01	0.07	<0.01	0.09					
Trial No.	L090397					28	pods**	<0.01	0.07	0.02	0.09					
GLP:	yes					28	seed	<0.01	<0.01	<0.01	<u><0.03</u>					
Year	2009					28	rest pl.[5]	0.02	5.25	0.61	5.53					
						34	pods*	<0.01	0.09	0.01	<u>0.10</u>					
						34	pods**	<0.01	0.09	0.03	0.12					
						34	seed	<0.01	<0.01	<0.01	<0.03					
						34	rest pl.[5]	0.01	4.77	0.61	5.07					
						41	pods**	<0.01	0.05	0.02	0.08					
						41	seed	<0.01	<0.01	<0.01	<0.03					
						41	rest pl.[5]	0.01	6.72	0.77	7.05					

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) at application
* with seeds
** without seeds
\# a further value was identified using Grubbs test at 99% probability and not taken into account for statistical evaluation
_ underlined values were used for MRL calculation; if higher residues were found and samplings later than the PHI of 28 days, those values were chosen

Table 6.3/43 Residues of bentazone in green beans after one application of BAS 351 45 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	370250	Green bean	France (South)	BAS 351 45 H: 1 x 1.2	55	0	plant[5]	92.13	0.65	0.63	93.34	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2009/1123296					28	pods*	<0.01	0.18	0.03	0.21	Bentazone				
Trial No.	L090400					28	pods**	<0.01	0.20	0.06	0.25	w. plant[5]	0.01-200	7	99	16
GLP:	yes					28	seed	<0.01	<0.01	<0.01	<0.03	pods*	0.01-0.10	4	96	7.9
Year	2009					28	rest pl.[5]	0.01	8.61	0.61	8.68	pods**	0.01-2.0	8	107	8.2
						35	pods*	<0.01	0.07	0.03	0.10	seed	0.01-0.10	6	106	7.1
						35	pods**	<0.01	0.21	0.06	0.26	rest pl.[5]	0.01-10	7	106	11
						35	seed	<0.01	<0.01	<0.01	<0.03	6-OH-bentazone				
						35	rest pl.[5]	<0.01	3.00	0.35	3.16	w. plant[5]	0.01-5.0	6[#]	93	15
						42	pods**	<0.01	0.31	0.10	0.40	pods*	0.01-0.20	4	105	10
						42	seed	<0.01	<0.01	<0.01	<0.03	pods**	0.01-1.0	6	108	4.4
						42	rest pl.[5]	<0.01	2.92	0.32	3.06	seed	0.01-0.10	6	103	10
Study code:	370250	Green bean	Italy	BAS 351 45 H: 1 x 1.2	55	0	plant[5]	105.25	0.95	0.30	106.42	rest pl.[5]	0.01-10	6	107	14
Doc ID:	2009/1123296					28	pods*	<0.01	0.04	0.01	0.06	8-OH-bentazone				
Trial No.	L090401					28	pods**	<0.01	0.06	0.02	0.09	w. plant[5]	0.01-5.0	7	109	20
GLP:	yes					28	seed	<0.01	<0.01	<0.01	<0.03	pods*	0.01-0.20	4	85	7.6
Year	2009					28	rest pl.[5]	0.01	4.64	0.66	4.99	pods**	0.01-1.0	6	102	12
						35	pods*	<0.01	0.05	0.02	0.08	seed	0.01-0.10	6	92	15
						35	pods**	<0.01	0.09	0.03	0.12	rest pl.[5]	0.01-10	6	104	11
						35	seed	<0.01	<0.01	<0.01	<0.03					
						35	rest pl.[5]	<0.01	4.08	0.52	4.33					
						42	pods**	<0.01	0.30	0.07	0.36					
						42	seed	<0.01	<0.01	<0.01	<0.03					
						42	rest pl.[5]	<0.01	2.95	0.43	3.19					

Table 6.3/43 Residues of bentazone in green beans after one application of BAS 351 45 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	370250	Green bean	Spain	BAS 351 45 H: 1 x 1.2	55	0	plant[5]	131.89	0.81	0.38	133.01					
Doc ID:	2009/1123296					28	rest pl.[5]	0.06	1.21	0.14	1.34					
Trial No.	L090402					36	pods*	<0.01	<0.01	<0.01	<u>0.03</u>					
GLP:	yes					36	rest pl.[5]	0.01	0.38	0.06	0.42					
Year	2009					42	pods**	<0.01	<0.01	<0.01	<0.03					
						42	seed	<0.01	<0.01	<0.01	<u>0.03</u>					
						42	rest pl.[5]	0.01	0.20	0.02	0.22					
Study code:	370250	Green bean	Greece	BAS 351 45 H: 1 x 1.2	55	0	plant[5]	100.55	0.45	0.33	101.28					
Doc ID:	2009/1123296					29	pods*	<0.01	0.02	<0.01	<u>0.04</u>					
Trial No.	L090403					29	pods**	<0.01	0.03	0.01	0.05					
GLP:	yes					29	seed	<0.01	0.02	<0.01	<u>0.04</u>					
Year	2009					29	rest pl.[5]	0.01	2.21	0.12	2.21					
						35	pods*	<0.01	0.01	<0.01	0.03					
						35	pods**	<0.01	0.03	0.01	0.05					
						35	seed	<0.01	<0.01	<0.01	<0.03					
						35	rest pl.[5]	<0.01	1.53	0.09	1.53					
						42	pods**	<0.01	0.04	0.02	0.07					
						42	seed	0.02	<0.01	<0.01	0.04					
						42	rest pl.[5]	<0.01	1.14	0.08	1.16					

Table 6.3/43 Residues of bentazone in green beans after one application of BAS 351 45 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	370250	Green bean	Spain	BAS 351 45 H: 1 x 1.2	55	0	plant[5]	77.50	0.86	0.36	78.65					
Doc ID:	2009/1123296					28	pods*	<0.01	0.01	<0.01	<u>0.03</u>					
Trial No.	L090482					28	pods**	<0.01	0.03	0.01	0.05					
GLP:	yes					28	seed	<0.01	<0.01	<0.01	<u><0.03</u>					
Year	2009					28	rest pl.[5]	0.04	4.74	0.38	4.86					
						35	pods*	<0.01	<0.01	<0.01	<0.03					
						35	pods**	<0.01	0.01	<0.01	0.03					
						35	seed	<0.01	<0.01	<0.01	<0.03					
						35	rest pl.[5]	0.02	3.45	0.46	3.69					
						42	pods**	<0.01	0.01	<0.01	0.03					
						42	seed	<0.01	<0.01	<0.01	<0.03					
						42	rest pl.[5]	0.02	3.47	0.39	3.64					

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) at application
* with seeds
** without seeds
_ underlined values were used for MRL calculation; if higher residues were found and samplings later than the PHI of 28 days, those values were chosen

Report:	II A 6.3.5/2 Schulz H. 2009(b) Study on the residue behaviour of Bentazone in green beans after treatment with BAS 351 45 H under field conditions in United Kingdom, Northern France, Southern France and Spain, 2008 BASF DocID 2009/1024806
Guidelines:	EEC 1607/VI/97 rev. 2 10.06.1999; EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8); EEC 7029/VI/95 rev. 5; EEC 7525/VI/95 rev. 7; SANCO/825/00 rev. 7 (17 March 2004); EEC 96/46 (16.07.1996)
GLP:	Yes (laboratory certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Executive Summary

During the 2008 growing season, 8 trials in green beans were conducted in Northern and Southern Europe to determine the residue level of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC). BAS 351 45 H (87% bentazone, SG) was applied once at a rate equivalent to 0.957 kg a.s./ha of bentazone. The application was done at crop growth stage BBCH 55. Specimens of whole plant without roots were collected at the day of the application. Specimens of pods with seeds and rest of plants without roots were sampled at 27-29 (BBCH 75-89), 35-36 (BBCH 77-89) and 40-42 (BBCH 77-89) days after the last application (DALA). Additionally, samples of seeds and pods without seeds were taken at the last two sampling times.
The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. Total bentazone residues in pods with seeds (green beans with pods) taken at 27-29, 35-36 and 40-42 DALA were <0.03-0.11, <0.03-0.08 and 0.03 mg/kg, respectively. No residues of bentazone or its metabolites above the limit of quantitation were found in any of the treated seed specimens (green beans without pods).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 45 H (SG)
Lot/Batch #:	2002-1, 87% bentazone, nominal
Purity:	Not reported
CAS#:	25057-89-0
Development code:	Not reported
Spiking levels:	

2. Test Commodity:

Crop:	Green bean
Type:	Legume vegetables
Variety:	Flamenco, Flavert, Valence, Flagoly, De Oro
Botanical name:	*Phaseolus vulgaris*
Crop parts(s) or processed commodity:	Whole plants without roots, pods with seeds, pods without seeds, seeds, rest of plants without roots
Sample size:	Min. 0.2-1.0 kg / 12-24 units

B. STUDY DESIGN

1. Test procedure

During the 2008 growing season, 8 trials in green beans were conducted in the United Kingdom, Northern and Southern France and Spain to determine the residue level of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
BAS 351 45 H (87% bentazone, SG) was applied once at a rate equivalent to 0.957 kg a.s./ha of bentazone and the spray volume used was 200 L/ha. The application was done at crop growth stage BBCH 55.
Specimens of whole plant without roots were collected at the day of the application. Specimens of pods with seeds and rest of plants without roots were sampled at 27-29 (BBCH 75-89), 35-36 (BBCH 77-89) and 40-42 (BBCH 77-89) days after the last application (DALA). Additionally, samples of seeds and pods without seeds were taken at the last two sampling times. Untreated control specimens were taken at every time point. Specimens were stored frozen (≤-18°C) until analysis. The maximum storage interval from harvest until analysis was 116 days.

Table 6.3/44 Target application rates and timings for green beans

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2008	8	1	F	BAS 351 45 H (SG)	BAS 351 H	0.957	200	BBCH 55

2. Description of analytical procedures

The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg.
Bentazone and its metabolites are extracted from plant or animal matrices with aqueous methanol. After purification of a 20% aliquot by an iso-octane liquid-liquid partition, the two relevant metabolites which are present as glucosides are hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic matrix constituents, a reversed phase C_{18}-SPE clean-up is performed. The final determination of bentazone and its hydroxyl-metabolites is performed by LC-MS/MS, monitoring two parent-daughter ion transitions for each compound.

II. RESULTS AND DISCUSSION

A summary of residues is given in Table 6.3/45. Details can be found in Table 6.3/46 and Table 6.3/47.
At 0 DALA, the total residues of bentazone in whole plant without roots ranged from 42.5 to 86.0 mg/kg. These residues degraded rapidly during the course of the study.
In pods with seeds taken at 27-29, 35-36 and 40-42 DALA, the total bentazone residues were <0.03-0.11, <0.03-0.08 and <0.03 mg/kg, respectively.
In pods without seeds taken at 35-36 and 40-42 DALA, the total bentazone residues were <0.03-0.11 and <0.03-0.14 mg/kg, respectively.
The total residues of bentazone in rest of plants ranged from 2.44-8.50 mg/kg in the specimens taken at 27-29 DALA. They decreased slightly to 2.84-5.90 mg/kg and 1.90-6.56 mg/kg at the subsequent sampling stages.
No residues of bentazone or its metabolites above the limit of quantitation were found in any of the treated seed specimens.
64 untreated specimens were analysed. Bentazone residues were found in 2 specimens (0.012 mg/kg in pods without seeds and 0.058 mg/kg in rest of plant without roots), 6-OH-bentazone residues were found in 8 specimens, mostly in rest of plant without roots, ranging from 0.010-3.78 mg/kg, and 8-OH-bentazone residues were found in 3 specimens (whole plant without root, pods with seeds and rest of plant without roots), ranging from 0.011-1.11 mg/kg. In some untreated specimens, unknown analyte contamination occurred, caused either during the field-specimen management or analytical phase.

Table 6.3/45 Summary of residues in green beans

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	2008	0	55	whole plant[5]	51.6-83.0	0.37-2.68	0.20-0.53	52.8-86.0
		27-29	**75-89**	**pods***	**<0.01**	**0.01-0.07**	**<0.01-0.01**	**0.03-0.09**
		35	77-89	pods*	<0.01	0.01-0.04	<0.01-0.02	0.03-0.07
		41-42	77-89	pods*	<0.01	0.01	<0.01	0.03
		27-29	75-89	rest plant[5]	<0.01-0.02	2.53-5.48	0.80-1.69	3.34-7.18
		35	77-89	rest plant[5]	<0.01-0.01	2.19-4.75	0.64-1.14	2.84-5.90
		41-42	77-89	rest plant[5]	<0.01-0.01	1.36-3.21	0.53-1.13	1.90-4.35
		35	77-83	pods**	<0.01	0.01-0.07	0.01-0.03	0.03-0.11
		41-42	77-89	pods**	<0.01	<0.01-0.09	<0.01-0.04	<0.03-0.14
		35	**77-83**	**seed**	**<0.01**	**<0.01**	**<0.01**	**<0.03**
		41-42	77-89	seed	<0.01	<0.01	<0.01	<0.03
EU South	2008	0	55	whole plant[5]	26.0-48.20	0.44-13.3	0.16-3.23	42.5-48.8
		27-28	**76-77**	**pods***	**<0.01**	**<0.01-0.08**	**<0.01-0.02**	**<0.03-0.11**
		35-36	79-81	pods*	<0.01	<0.01-0.05	<0.01-0.02	<0.03-0.08
		27-28	76-77	rest plant[5]	<0.01-0.04	1.86-6.79	0.57-1.67	2.44-8.50
		35-36	79-81	rest plant[5]	<0.01-0.08	3.53-4.46	0.99-1.26	4.53-5.76
		40-42	81-85	rest plant[5]	<0.01-0.01	2.68-5.03	0.93-1.62	3.62-6.56
		35-36	79-81	pods**	<0.01	<0.01-0.08	<0.01-0.02	<0.03-0.11
		40-42	81-85	pods**	<0.01	<0.01-0.10	<0.01-0.03	<0.03-0.14
		35-36	**79-81**	**seed**	**<0.01**	**<0.01**	**<0.01**	**<0.03**
		40-42	81-85	seed	<0.01	<0.01	<0.01	<0.03

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) without roots
* with seeds
** without seeds
Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Total bentazone residues in pods with seeds (green beans with pods) taken at 27-29, 35-36 and 40-42 DALA, the total bentazone residues were <0.03-0.11, <0.03-0.08 and <0.03 mg/kg, respectively. No residues of bentazone or its metabolites were found above the limit of quantitation in any of the treated seed specimens (green beans without pods).

Table 6.3/46 Residues of bentazone in green beans after one application of BAS 351 45 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	347918	Green bean	United Kingdom	BAS 351 45 H: 1 x 0.957	55	0	plant[5]	51.60	2.68	0.53	54.81	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2009/1024806					27	pods*	<0.01	0.05	0.01	0.07	Bentazone				
Trial No.	L080310					27	rest pl.[5]	0.02	2.87	0.92	3.81	w. plant[5]	0.01-1.0	6	92	5.7
GLP:	yes					35	pods*	<0.01	0.04	0.01	0.06	pods*	0.01-1.0	12	84	7.6
Year	2008					35	pods**	<0.01	0.01	0.01	0.03	pods**	0.01-1.0	11	86	6.6
						35	seed	<0.01	<0.01	<0.01	<0.03	seed	0.01-1.0	9	88	9.3
						35	rest pl.[5]	0.01	2.21	0.70	2.92	rest pl.[5]	0.01-1.0	12	83	11
						41	pods**	<0.01	<0.01	<0.01	<0.03	6-OH-bentazone				
						41	seed	<0.01	<0.01	<0.01	<0.03	w. plant[5]	0.01-1.0	6	100	5.3
						41	rest pl.[5]	<0.01	1.36	0.53	1.90	pods*	0.01-1.0	12	100	8.2
Study code:	347918	Green bean	United Kingdom	BAS 351 45 H: 1 x 0.957	55	0	plant[5]	83.00	2.57	0.45	86.02	pods**	0.01-1.0	11	93	11
Doc ID:	2009/1024806					27	pods*	<0.01	0.07	0.01	0.09	seed	0.01-1.0	9	93	12
Trial No.	L080311					27	rest pl.[5]	0.02	5.48	1.69	7.18	rest pl.[5]	0.01-1.0	12	98	5.5
GLP:	yes					35	pods*	<0.01	0.04	0.02	0.07	8-OH-bentazone				
Year	2008					35	pods**	<0.01	0.04	0.02	0.07	w. plant[5]	0.01-1.0	6	99	6.5
						35	seed	<0.01	<0.01	<0.01	<0.03	pods*	0.01-1.0	12	86	12
						35	rest pl.[5]	0.01	4.00	1.13	5.14	pods**	0.01-1.0	11	87	8.7
						41	pods**	<0.01	0.04	0.01	0.06	seed	0.01-1.0	9	89	14
						41	seed	<0.01	<0.01	<0.01	<0.03	rest pl.[5]	0.01-1.0	12	85	12
						41	rest pl.[5]	0.01	3.21	1.13	4.35					

Table 6.3/46 Residues of bentazone in green beans after one application of BAS 351 45 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	347918	Green bean	France (North)	BAS 351 45 H: 1 x 0.957	55	0	plant[5]	56.60	0.37	0.20	57.17					
Doc ID:	2009/1024806					27	pods*	<0.01	0.02	0.01	0.04					
Trial No.	L080312					27	rest pl.[5]	<0.01	3.15	0.88	4.04					
GLP:	yes					35	pods*	<0.01	0.03	0.01	<u>0.05</u>					
Year	2008					35	pods**	<0.01	0.07	0.03	0.11					
						35	seed	<0.01	<0.01	<0.01	<u><0.03</u>					
						35	rest pl.[5]	<0.01	4.75	1.14	5.90					
						42	pods**	<0.01	0.09	0.04	0.14					
						42	seed	<0.01	<0.01	<0.01	<0.03					
						42	rest pl.[5]	<0.01	2.96	0.91	3.87					
Study code:	347918	Green bean	France (North)	BAS 351 45 H: 1 x 0.957	55	0	plant[5]	53.60	1.17	0.45	55.22					
Doc ID:	2009/1024806					29	pods*	<0.01	0.01	<0.01	<u>0.03</u>					
Trial No.	L080313					29	rest pl.[5]	<0.01	2.53	0.80	3.34					
GLP:	yes					35	pods*	<0.01	0.01	<0.01	0.03					
Year	2008					35	rest pl.[5]	<0.01	2.19	0.64	2.84					
						42	pods*	<0.01	0.01	<0.01	0.03					
						42	rest pl.[5]	<0.01	1.99	0.62	2.62					

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) at application
* with seeds
** without seeds
_ underlined values were used for MRL calculation; if higher residues were found and samplings later than the PHI of 28 days, those values were chosen

Table 6.3/47 Residues of bentazone in green beans after one application of BAS 351 45 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	347918	Green bean	France (South)	BAS 351 45 H: 1 x 0.957	55	0	plant[5]	48.20	0.44	0.16	48.80	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2009/1024806					27	pods*	<0.01	0.08	0.02	0.11	Bentazone				
Trial No.	L080314					27	rest pl. [5]	0.01	3.96	1.25	5.22	w. plant[5]	0.01-1.0	6	92	5.7
GLP:	yes					36	pods*	<0.01	0.05	0.02	0.08	pods*	0.01-1.0	12	84	7.6
Year	2008					36	pods**	<0.01	0.08	0.02	0.11	pods**	0.01-1.0	11	86	6.6
						36	seed	<0.01	<0.01	<0.01	<0.03	seed	0.01-1.0	9	88	9.3
						36	rest pl. [5]	<0.01	3.53	0.99	4.53	rest pl. [5]	0.01-1.0	12	83	11
						41	pods**	<0.01	0.10	0.03	0.14	6-OH-bentazone				
						41	seed	<0.01	<0.01	<0.01	<0.03	w. plant[5]	0.01-1.0	6	100	5.3
						41	rest pl. [5]	0.01	2.68	0.93	3.62	pods*	0.01-1.0	12	100	8.2
Study code:	347918	Green bean	France (South)	BAS 351 45 H: 1 x 0.957	55	0	plant[5]	46.80	0.48	0.23	47.50	pods**	0.01-1.0	11	93	11
Doc ID:	2009/1024806					27	pods*	<0.01	0.04	0.01	0.06	seed	0.01-1.0	9	93	11
Trial No.	L080315					27	rest pl. [5]	<0.01	4.35	1.39	5.75	rest pl. [5]	0.01-1.0	12	98	5.5
GLP:	yes					35	pods*	<0.01	0.03	0.01	0.05	8-OH-bentazone				
Year	2008					35	pods**	<0.01	0.03	0.01	0.05	w. plant[5]	0.01-1.0	6	99	6.5
						35	seed	<0.01	<0.01	<0.01	<0.03	pods*	0.01-1.0	12	86	12
						35	rest pl. [5]	<0.01	3.56	1.08	4.65	pods**	0.01-1.0	11	87	8.7
						40	pods**	<0.01	0.01	<0.01	0.03	seed	0.01-1.0	9	89	14
						40	seed	<0.01	<0.01	<0.01	<0.03	rest pl. [5]	0.01-1.0	12	85	12
						40	rest pl. [5]	<0.01	4.65	1.62	6.28					

Table 6.3/47 Residues of bentazone in green beans after one application of BAS 351 45 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	347918	Green bean	Spain	BAS 351 45 H: 1 x 0.957	55	0	plant[5]	26.00	13.30	3.23	42.52					
Doc ID:	2009/1024806					28	pods*	<0.01	<0.01	<0.01	<u>0.03</u>					
Trial No.	L080316					28	rest pl.[5]	<0.01	1.86	0.57	2.44					
GLP:	yes					35	pods*	<0.01	<0.01	<0.01	<0.03					
Year	2008					35	pods**	<0.01	<0.01	<0.01	<0.03					
						35	seed	<0.01	<0.01	<0.01	<u>0.03</u>					
						35	rest pl.[5]	0.04	4.46	1.26	5.76					
						42	pods**	<0.01	<0.01	<0.01	<0.03					
						42	seed	<0.01	<0.01	<0.01	<0.03					
						42	rest pl.[5]	0.01	4.24	1.20	5.45					

Table 6.3/47 Residues of bentazone in green beans after one application of BAS 351 45 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	347918	Green bean	Spain	BAS 351 45 H: 1 x 0.957	55	0	plant[5]	36.00	9.86	1.38	47.24					
Doc ID:	2009/1024806					28	pods*	<0.01	<0.01	<0.01	<u>0.03</u>					
Trial No.	L080317					28	rest pl.[5]	0.04	6.79	1.67	8.50					
GLP:	yes					35	pods*	<0.01	<0.01	<0.01	<0.03					
Year	2008					35	pods**	<0.01	<0.01	<0.01	<0.03					
						35	seed	<0.01	<0.01	<0.01	<u>0.03</u>					
						35	rest pl.[5]	0.08	3.83	1.13	5.04					
						42	pods**	<0.01	<0.01	<0.01	<0.03					
						42	seed	<0.01	<0.01	<0.01	<0.03					
						42	rest pl.[5]	<0.01	5.03	1.52	6.56					

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) at application
* with seeds
** without seeds
_ underlined values were used for MRL calculation; if higher residues were found and samplings later than the PHI of 28 days, those values were chosen

Peas

Report:	II A 6.3.5/3 Oxspring S. 2008(g) Study on the residue behaviour of Bentazone in fresh and dried peas after treatment with BAS 351 45 H under field conditions in Northern and Southern Europe during 2007 BASF DocID 2008/1049972
Guidelines:	None
GLP:	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

During the 2007 growing season, four field trials were conducted in representative fresh and dried pea growing areas in France (dried peas) and the United Kingdom (fresh peas) to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone (BH 351-6-OH) and 8-OH-bentazone (BH 351-8-OH) in or on raw agricultural commodities (RAC). BAS 351 45 H, an SG formulation of bentazone (87% w/w), was foliar applied once at a rate equivalent to 0.957 kg a.s./ha to fresh and dried peas at 28 days before expected harvest. Specimens of whole plant (without roots or pods) and whole pods were collected immediately after the application had dried (0 DALA) from each plot, as well as seeds that had been mechanically harvested and seeds and whole pods that were manually harvested 28 days after application. In the following, only the information on the fresh pea trials is given. Details on dry peas are presented in chapter 6.3.16.
Residues were determined by means of BASF Method No. 438/2; the limit of quantitation was 0.01 mg/kg for all analytes. At 28 days after application, no residues of bentazone or its metabolites were detected above the limit of quantitation (<0.01 mg/kg) in any of the green seed specimens. Similarly, whole pod specimens showed no residues above the limit of quantitation for bentazone and 8-OH-bentazone and levels of only 0.02 to 0.03 mg/kg for 6-OH-bentazone. No differences were noted between mechanically and manually harvest techniques.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 351 45 H
Lot/Batch #: 2002-1, 87% bentazone, nominal
Purity: Not reported
CAS#: 25057-89-0
Development code: Not reported
Spiking levels: 0.01-5.0 mg/kg

2. Test Commodity:

Crop: Pea
Type: Legume vegetables
Variety: Fresh peas: Swallow, Waverx
Botanical name: *Pisum sativum*
Crop parts(s) or processed commodity: Whole plant without root or pods, whole pods (mechanically harvested), seed (mechanically and manually harvested)
Sample size: Min. 1 kg / 12-24 units (nominal)

B. STUDY DESIGN

1. Test procedure

During the 2007 growing season, four field trials were conducted in representative fresh and dried pea growing areas in France (dried peas) and the UK (fresh peas) to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone (BH 351-6-OH) and 8-OH-bentazone (BH 351-8-OH) in or on raw agricultural commodities (RAC).

BAS 351 45 H, an SG formulation of bentazone (87% w/w), was foliar applied once at a rate equivalent to 0.957 kg a.s./ha to fresh and dried peas at 28 days before expected harvest. The nominal spray volume used was 200 L/ha.

Specimens of whole plant (without roots or pods) and whole pods were collected immediately after the application had dried (0 DALA) from each plot, as well as seeds that had been mechanically harvested and seeds and whole pods that were manually harvested 28 days after application. However, at Trials AF/12152/BAI1 and 2 (L070800 and L070801) the fresh peas were not mature enough to sample whole pods at the 0 DALA sampling timing.

In the following, only the information on the fresh pea trials is given. Details on dry peas are presented in chapter 6.3.16.

Table 6.3/48 Target application rates and timings for fresh peas

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2007	2	1	F	BAS 351 45 H (SG)	BAS 351 H	0.957	200	28±1 days before harvest

2. Description of analytical procedures

All samples were analysed according to BASF Method No. 438/2. Bentazone, 6-OH-bentazone and 8-OH-bentazone are extracted from plant material using a mixture of methanol and water. The two metabolites that were present as glucosides were hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$ precipitation step to remove acidic plant constituents, a reversed phase C_{18} column clean-up was performed or the extract was filtered and one aliquot taken, evaporated to dryness and dissolved in a methanol/water solution. The final determination of the residues of bentazone and its hydroxyl metabolites was performed by LC-MS/MS. The limit of quantitation was 0.01 mg/kg for all substances.

II. RESULTS AND DISCUSSION

The residue ranges in peas determined at DALAs between 0 and 28 days are presented in Table 6.3/49. Details can be found in Table 6.3/50.
Directly after the application residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone ranged from 5.01 to 6.24 mg/kg in whole plants without root or pods. Bentazone accounted for 4.28 to 4.85 mg/kg of the total residue, while 6-OH-bentazone and 8-OH-bentazone could be detected at levels of between 0.16 and 1.96 mg/kg and <0.01 and 0.13 mg/kg respectively. At 28 days after application residue levels decreased significantly. No residues of bentazone or its metabolites were detected above the limit of quantification (<0.01 mg/kg) in any of the seed specimens. Similarly, whole pod specimens showed no residues above the limit of quantification for bentazone and 8-OH-bentazone and levels of only 0.02 to 0.03 mg/kg for 6-OH-bentazone.

Table 6.3/49 Summary of residues in green peas

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	2007	0	51-65	whole plant[5]	4.28-4.85	0.16-1.96	<0.01-0.13	5.01-6.24
		28	**79-85**	**seed***	**<0.01**	**<0.01**	**<0.01**	**<0.03**
		28	**79-85**	**seed****	**<0.01**	**<0.01**	**<0.01**	**<0.03**
		28	**79-85**	**whole pods****	**<0.01**	**0.02-0.03**	**<0.01**	**0.04-0.05**

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) without root or pods
* mechanically harvested
** manually harvested
Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

At 28 days after application, no residues of bentazone or its metabolites were detected above the limit of quantitation (<0.01 mg/kg) in any of the seed specimens. Similarly, whole pod specimens showed no residues above the limit of quantitation for bentazone and 8-OH-bentazone and levels of only 0.02 to 0.03 mg/kg for 6-OH-bentazone. No differences were noted between mechanically and manually harvest techniques.

Table 6.3/50 Residues of bentazone in green peas after one application of BAS 351 45 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	303257	Green pea	United Kingdom	BAS 351 45 H: 1 x 0.957	51-59	0	plant[5]	4.85	0.16	<0.01	5.01	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2008/1049972					28	seed*	<0.01	<0.01	<0.01	<u>≤0.03</u>	Bentazone				
Trial No.	L070800					28	seed**	<0.01	<0.01	<0.01	<0.03	seeds/pods	0.01-1.0	7	88	14
GLP:	yes					28	wh. pod**	<0.01	0.02	<0.01	<u>0.04</u>	w. plant[5]	0.01-5.0	6	86	10
Year:	2007											6-OH-bentazone				
Study code:	303257	Green pea	United Kingdom	BAS 351 45 H: 1 x 0.957	55-65	0	plant[5]	4.28	1.96	0.13	6.24	seeds/pods	0.01-5.0	9	92	14
Doc ID:	2008/1049972					28	seed*	<0.01	<0.01	<0.01	<u>≤0.03</u>	w. plant[5]	0.01-1.0	4	84	5
Trial No.	L070801					28	seed**	<0.01	<0.01	<0.01	<0.03	8-OH-bentazone				
GLP:	yes					28	wh. pod**	<0.01	0.03	<0.01	<u>0.05</u>	seeds/pods	0.01-1.0	7	83	7
Year:	2007											w. plant[5]	0.01-5.0	6	82	6

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots or pods
6) at application
* mechanically harvested
** manually harvested
_ underlined values were used for MRL calculation

6.3.6 Pulses dry

Table 6.3/51 GAP on BAS 351 H for residue trials in/on pulses

Crop	Maximum Applied Dose	Water Volume	PHI	Application Method	Application Timing
Bean	1 x 0.96 kg BAS 351 H/ha	200-400 L/ha	F	spray application	after BBCH 14
Pea	1 x 0.96 kg BAS 351 H/ha	200-400 L/ha	F	spray application	BBCH 11-12

PHI = pre-harvest interval

Table 6.3/52 GAP information of residue trials conducted in dried beans between 1999 and 2010

Region	Country	Formulation	Application[0]				DALA[1]
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
EU North	United Kingdom (1 trial) France (1 trial)	BAS 351 45 H, SG	spray application	0.957	0.479	1	0 28
	The Netherlands (1 trial) France (2 trials)	BAS 351 32 H, SL	spray application	1.552	0.517	1	12-31 48-68 82-101
	Germany (2 trials)	BAS 351 32 H, SL	spray application	1.536	0.512	1	20-25 47-51 89-98
EU South	France (2 trials)	BAS 351 45 H, SG	spray application	0.957	0.479	1	0 28
	France (2 trials) Spain (2 trials)	BAS 351 32 H, SL	spray application	1.55-1.67	0.512	1	18-27 48-52 67-88
	France (2 trials) Spain (2 trials)	BAS 351 32 H, SL	spray application	1.552	0.517	1	17-24 52-77 66-107
	Spain (1 trial) France (1 trial)	BAS 351 45 H, SG	spray application	1.20	0.60	1	0 122-204

0) actual application rates varied by 10% at most
1) days after last application

Table 6.3/53 GAP information of residue trials conducted in dried peas in 2007

Region	Country	Formulation	Application[0]				DALA[1]
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
EU South	France (2 trials)	BAS 351 45 H, SG	spray application	0.957	0.479	1	0 28

0) actual application rates varied by 10% at most
1) days after last application

Beans

Report: II A 6.3.6/1
Schroth E., Martin T. 2011(a)
Study on the residue behavior of BAS 351 H (Bentazone) in broad bean (Vicia faba) after the application of BAS 351 45 H under field conditions in France (South) and Spain, 2009-2010
BASF DocID 2011/1059498

Guidelines: EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8); EEC 1607/VI/97 rev. 2 10.06.1999; EEC 7029/VI/95 rev. 5; EEC 7525/VI/95 rev. 7

GLP: Yes
(laboratory certified by ENAC, Entidad Nacional de Acreditación, Madrid Spain)

Executive Summary

During the 2009/2010 growing season, two field trials in broad bean were conducted in representative growing areas in Southern Europe to determine the residue level of bentazone (BAS 351 45 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC). BAS 351 45 H, an SG formulation of bentazone, was applied once at a rate equivalent to 1.20 kg a.s./ha at crop growth stage BBCH 14-15. Specimens of whole plant (without roots) were collected at the day of the application (0 DALA) and specimens of rest of plant without roots and seed were collected at BBCH 89 (harvest), 122-204 days after the application.
Specimens were analysed using BASF Method No. 438/2 (L0044/02) to quantify the residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone. The limit of quantitation (LOQ) of the method was 0.01 mg/kg for each analyte. The total bentazone residues in broad bean seeds were <0.03 mg/kg at harvest (BBCH 89).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 351 45 H (SG)
Lot/Batch #: 2002-1, 87% nominal
Purity: Not reported
CAS#: 25057-89-0
Development code: Not reported
Spiking levels: 0.01-80 mg/kg

2. Test Commodity:

Crop: Broad bean
Type: Pulses
Variety: Luz de Otoño, Castel
Botanical name: *Vicia faba*
Crop parts(s) or processed commodity: Whole plants without roots, rest of plant without roots, seed
Sample size: 0.5-1 kg / 12 units (nominal)

B. STUDY DESIGN

1. Test procedure

During the 2009/2010 growing season, two field trials in broad bean were conducted in representative growing areas in Southern France and Spain to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).

BAS 351 45 H, an SG formulation of bentazone (87% w/w), was applied once at a rate equivalent to 1.20 kg a.s./ha at crop growth stage BBCH 14-15. The spray volume used was 200 L/ha.

Specimens of whole plant (without roots) were collected at the day of the application (0 DALA) and specimens of rest of plant without roots and seed were collected at BBCH 89 (harvest), 122-204 days after the application.

Untreated control specimens were taken at every time point. All specimens were stored frozen (≤-18°C) until analysis. The maximum storage interval from harvest until analysis was 538 and 334 days for whole plant and seed samples, respectively.

Table 6.3/54 Target application rates and timings for dried beans

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2009/2010	2	1	F	BAS 351 45 H (SG)	Bentazone	1.20	200	BBCH 11-15

2. Description of analytical procedures

Specimens were analysed using BASF Method No. 438/2 (L0044/02) to quantify the residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone. Residues were extracted from plant matrices using a mixture of methanol and water. The two metabolites that were present as glucosides were hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$ precipitation step to remove acidic plant constituents, a reversed phase C_{18}-column clean-up was performed. The final determination of the residues of bentazone and its hydroxyl metabolites was performed by LC-MS/MS. The limit of quantitation (LOQ) of the method was 0.01 mg/kg for each analyte.

II. RESULTS AND DISCUSSION

The residue ranges are presented in Table 6.3/58. Details can be found in Table 6.3/59.
The parent bentazone residues in broad bean whole plant without roots specimens taken directly after the application ranged between 45-65 mg/kg. At harvest (BBCH 89), residues were below the LOQ (<0.01 mg/kg) in rest of plant without roots and also in seeds.
The 6-OH-bentazone residues in broad bean whole plant without roots specimens taken directly after the application ranged between 0.65-0.75 mg/kg. At harvest (BBCH 89), residues were below the LOQ (<0.01 mg/kg) in rest of plant without roots and also in seeds.
The 8-OH-bentazone residues in broad bean whole plant without roots specimens taken directly after the application ranged between 0.62-0.52 mg/kg. At harvest (BBCH 89), residues were below the LOQ (<0.01 mg/kg) in rest of plant without roots and also in seeds.
The total bentazone residues in broad bean whole plant without roots specimens taken directly after the application ranged between 46.27-66.27 mg/kg. At harvest (BBCH 89), residues were <0.03 mg/kg in rest of plant without roots and also in seeds.
No residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone above the LOQ (<0.01 mg/kg) were found in the control specimens of this study.

Table 6.3/55 Summary of residues in dried beans

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU South	2009/2010	0	14-15	whole plants[5]	45-65	0.65-0.75	0.52-0.62	46.27-66.27
		122-204	89	rest plants[5]	<0.01	<0.01	<0.01	<0.03
		122-204	**89**	**seed**	**<0.01**	**<0.01**	**<0.01**	**<0.03**

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) without roots

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

The total bentazone residues in broad bean seeds (RAC) were <0.03 mg/kg at harvest (BBCH 89).

Table 6.3/56 Residues of bentazone in dried beans after one application of BAS 351 45 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[2]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	370251	Broad bean	Spain	BAS 351 45 H: 1 x 1.20	15	0	plant[5]	45	0.65	0.62	46.27	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2011/1059498					204	rest pl.[5]	<0.01	<0.01	<0.01	<0.03	Bentazone				
Trial No.	L090404					204	seed	<0.01	<0.01	<0.01	<u>0.03</u>	plant[5]	0.01-80	4	98	7.9
GLP:	yes											seeds	0.01-0.10	2	95	N/A
Year:	2009/2010											6-OH-bentazone				
Study code:	370251	Broad bean	France (South)	BAS 351 45 H: 1 x 1.20	14-15	0	plant[5]	65	0.75	0.52	66.27	plant[5]	0.01-1	3	97	6.2
Doc ID:	2011/1059498					122	rest pl.[5]	<0.01	<0.01	<0.01	<0.03	seeds	0.01-0.10	2	78	N/A
Trial No.	L090405					122	seed	<0.01	<0.01	<0.01	<u>0.03</u>	8-OH-bentazone				
GLP:	yes											plant[5]	0.01-1	3	91	8.2
Year:	2009/2010											seeds	0.01-0.10	2	80	N/A

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as parent equivalent, conversion factor 0.938
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) at application
_ underlined values were used for MRL calculation
N/A not applicable

Report:	II A 6.3.6/2 Schulz H. 2002(c) Determination of the residues of BAS 351 H in beans following treatment with BAS 351 32 H (Basagran) under field conditions in Southern France and Spain 2000 BASF DocID 2002/1006296
Guidelines:	Guidelines on Producing Pesticide Residue Data from Supervised Trials FAO Rome 1990; BBA IV 3-3; IVA Guidelines for Residue Studies Sections IA and IB 2nd edition 1992
GLP:	Yes (laboratory certified by Hessisches Ministerium fuer Umwelt, Landwirtschaft und Forsten, Wiesbaden)

Executive Summary

During the growing season of 2000, four supervised residue trials on bush beans and stick beans were conducted in Southern Europe to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC). A single application with the formulated product BAS 351 32 H (480 g/L, SL) was performed. The nominal application rates ranged from 1.536-1.680 kg a.s./ha. The application was done at a crop growth stage of BBCH 12-13. Specimens of whole plants without roots were taken 18-27 days after the application (DALA) (BBCH 29-51); pods with seed and rest plants were sampled at 48-52 DALA (BBCH 79) and dry beans without pods were collected at 67-88 DALA (BBCH 85-97).
All samples were analysed for residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone according to BASF Method No. 438/1 with a limit of quantitation of 0.02 mg/kg for each analyte. Total bentazone residues in dry beans were at or below the LOQ of 0.06 mg/kg at 67-88 days after the application (BBCH 85-97). Therefore, the residue values were used for MRL determination (see chapter 6.7) even though an exaggerated application rate compared to the critical GAP was used.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 351 32 H (SL)
Lot/Batch #: 2000-1; bentazone 480 g/L (nominal)
Purity: Not reported
CAS#: 25057-89-0
Development code: Not reported
Spiking levels: 0.02-2.0 mg/kg

2. Test Commodity:

Crop: Bean (dry)
Type: Pulses
Variety: Coco Blanc Gautier, Langue de feu, B.B.L., Garrafal Oro
Botanical name: *Phaseolus vulgaris*
Crop parts(s) or processed commodity: Whole plants without roots, pods with seeds, rest of plants, dry beans without pods
Sample size: Min. 0.5-1 kg

B. STUDY DESIGN

1. Test procedure

During the growing season of 2000, four supervised residue trials on bush beans and stick beans were conducted in Southern France and Spain to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).

A single application with the formulated product BAS 351 32 H (480 g/L, SL) was performed. The nominal application rates ranged from 3.2-3.5 L product /ha, equivalent to 1.536-1.680 kg a.s./ha. The application was done at a crop growth stage of BBCH 12-13. The spray volume used was 300-330 L/ha.

Specimens of whole plants without roots were taken 18-27 days after the application (DALA) (BBCH 29-51); pods with seed and rest plants were sampled at 48-52 DALA (BBCH 79) and dry beans without pods were collected at 67-88 DALA (BBCH 85-97).

Table 6.3/57 Target application rates and timings for dried beans

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2000	4	1	F	BAS 351 32 H (SL)	Bentazone	1.536	300	BBCH 12-13

2. Description of analytical procedures

All samples were analysed for residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone according to BASF Method No. 438/1 with a limit of quantitation of 0.02 mg/kg for each analyte.

Bentazone and its metabolites were extracted from plant matrices with aqueous methanol. After purification by an iso-octane liquid-liquid partition, the two relevant metabolites which are present as glucosides were hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic plant constituents, a reversed phase C_{18}-column clean-up was performed. The analytes were then methylated with diazomethane and their derivatives were purified using a silica gel-column. The final determination of the residues of bentazone and its hydroxy metabolites was performed by GC-MS.

II. RESULTS AND DISCUSSION

The residue ranges are presented in Table 6.3/61. Details can be found in Table 6.3/62.
18-27 days after the application, the bentazone residues ranged from 0.0474-0.194 mg/kg. A decline of the residues was observed at the following two sampling dates. At 48-52 DALA, in pods with seeds and in rest plant, the bentazone residues were <0.02 and <0.02-0.027 mg/kg, respectively. In one out of four dry bean samples, the bentazone residue was found slightly above the LOQ (0.021 mg/kg).
Regarding the two metabolites 6-OH-bentazone and 8-OH-bentazone, at 18-27 DALA, the residues ranged from 0.417-1.705 mg/kg and from 0.13-0.362 mg/kg, respectively. A decline of these residues was observed at the following two sampling dates. No residues above the LOQ were found in the pods with seeds at 48-52 DALA. In the corresponding rest plant samples, the residues ranged from <0.02-0.045 and <0.02-0.041 mg/kg, respectively. In dry beans at 67-88 DALA, no residues of 6-OH-bentazone above the LOQ were observed. In one out of four dry bean samples, the 8-OH-bentazone residue were above the LOQ (0.023 mg/kg).
No residues above the LOQ of 0.02 mg/kg of bentazone and metabolites were detected in any of the untreated control specimens.

Table 6.3/58 Summary of residues in dried beans

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU South	2000	18-27	29-51	plant[5]	0.047-0.194	0.417-1.705	0.13-0.362	0.56-2.08
		48-52	79	pods*	<0.02	<0.02	<0.02	<0.06
		48-52	79	rest plant	<0.02-0.027	<0.02-0.045	<0.02-0.041	0.07-0.08
		67-88	**85-97**	**dry beans****	**<0.02-0.021**	**<0.02**	**<0.02-0.023**	**<0.06-0.06**

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) without root
* with seeds
** without pods
Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Total bentazone residues in dry beans were at or below the LOQ of 0.06 mg/kg at 67-88 days after the application (BBCH 85-97).

Table 6.3/59 Residues of bentazone in dried beans after one application of BAS 351 32 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	IF-100/09102-00	Bush bean	France (South)	BAS 351 32 H: 1 x 1.669	12	21	plant[5]	0.194	0.752	0.148	1.04	**Method:** No. 438/1; LOQ = 0.02 mg/kg				
Doc ID:	2002/1006296					48	pods*	<0.02	<0.02	<0.02	<0.06	Bentazone				
Trial No.	X006207					48	rest plant	0.027	0.03	0.025	0.08	plant[5]	0.02-2.0	4	84	4.6
GLP:	yes					67	beans**	<0.02	<0.02	<0.02	<0.06	pods*	0.02-2.0	4	87	11
Year:	2000											rest plant	0.02-2.0	4	93	10
Study code:	IF-100/09102-00	Stick bean	France (South)	BAS 351 32 H: 1 x 1.546	12-13	27	plant[5]	0.141	1.705	0.362	2.08	beans**	0.02-2.0	4	105	8.3
Doc ID:	2002/1006296					52	pods*	<0.02	<0.02	<0.02	<0.06	6-OH-bentazone				
Trial No.	X006208					52	rest plant	<0.02	<0.02	0.041	0.08	plant[5]	0.02-2.0	4	72	6.8
GLP:	yes					69	beans**	0.021	<0.02	0.023	0.06	pods*	0.02-2.0	4	75	8.6
Year:	2000											rest plant	0.02-2.0	4	87	9.3
Study code:	IF-100/09102-00	Bush bean	Spain	BAS 351 32 H: 1 x 1.587	12	18	plant[5]	0.0474	0.417	0.13	0.56	beans**	0.02-2.0	4	83	2.7
Doc ID:	2002/1006296					48	pods*	<0.02	<0.02	<0.02	<0.06	8-OH-bentazone				
Trial No.	00S035R					48	rest plant	<0.02	0.031	<0.02	0.07	plant[5]	0.02-2.0	4	80	15
GLP:	yes					88	beans**	<0.02	<0.02	<0.02	<0.06	pods*	0.02-2.0	4	88	18
Year:	2000											rest plant	0.02-2.0	4	92	19

Table 6.3/59 Residues of bentazone in dried beans after one application of BAS 351 32 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	IF-100/09102-00	Stick bean	Spain	BAS 351 32 H: 1 x 1.546	12-13	18	plant[5]	0.09	1.033	0.202	1.24	beans**	0.02-2.0	4	86	15
Doc ID:	2002/1006296					48	pods*	<0.02	<0.02	<0.02	<0.06					
Trial No.	00S036R					48	rest plant	<0.02	0.045	<0.02	0.08					
GLP:	yes					88	beans**	<0.02	<0.02	<0.02	<u>0.06</u>					
Year:	2000															

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.02 + 0.02 + 0.02 = 0.06; 0.02 + <0.02 + <0.02 = 0.06; <0.02 + <0.02 + <0.02 = <0.06
5) without roots
6) at application
* with seeds
** dry without pods
_ underlined values were used for MRL calculation

Report:	II A 6.3.6/3 Blaschke U.G. 2001(e) Determination of the magnitude of the residue of BAS 351 32 H in/on bean raw agricultural commodity specimens from supervised field trials in Northern and Southern Europe in 1999 BASF DocID 2001/1000926
Guidelines:	FAO Guidelines Rome 1990; EEC 96/68; EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8)
GLP:	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

During the growing season of 1999, seven supervised residue trials on pole beans and bush beans were conducted in Northern and Southern Europe to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC). A single application with the formulated product BAS 351 32 H (480 g/L, SL) was performed. The nominal application rate was 1.552 kg a.s./ha. The application was done at a crop growth stage of BBCH 12-13. Specimens of shoots were taken 12-31 days after the application (DALA) (BBCH 22-32); pods were sampled at 48-77 DALA (BBCH 79). Seed and straw specimens were obtained upon crop maturity (66-107 DALA, BBCH 79-89).
All samples were analysed for residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone according to BASF Method No. 438/1 with a limit of quantitation of 0.02 mg/kg for each analyte. Total bentazone residues in dry beans (seeds) were at or below the LOQ of 0.06 mg/kg at 66-107 days after the application (BBCH 79-89). Therefore, the residue values were used for MRL determination (see chapter 6.7) even though an exaggerated application rate compared to the critical GAP was used.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 32 H (SL)
Lot/Batch #:	98-1; bentazone 480 g/L (nominal)
Purity:	Not reported
CAS#:	25057-89-0
Development code:	Not reported
Spiking levels:	0.02-2.0 mg/kg

2. Test Commodity:

Crop:	Bean (dry)
Type:	Pulses
Variety:	Berna, Flagrano, Phenomene, Novirex, Hiltrud, BBL-274, Garrafal, Oro
Botanical name:	*Phaseolus vulgaris*
Crop parts(s) or processed commodity:	Shoots, pods, seeds, straw
Sample size:	0.5-1 kg (nominal)

B. STUDY DESIGN

1. Test procedure

During the growing season of 1999, seven supervised residue trials on pole beans and bush beans were conducted in the Netherlands, Northern and Southern France and Spain to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
A single application with the formulated product BAS 351 32 H (480 g/L, SL) was performed. The nominal application rate was 3.2 L product/ha, equivalent to 1.552 kg a.s./ha (actual: 1.518-1.617 kg a.s./ha). The application was done at a crop growth stage of BBCH 12-13. The spray volume used was 293-313 L/ha.
Specimens of shoots were taken 12-31 days after the application (DALA) (BBCH 22-32); pods were sampled at 48-77 DALA (BBCH 79). Seed and straw specimens were obtained upon crop maturity (66-107 DALA, BBCH 88-89). From the pole bean site in Spain (BSF/608-7), another set of bean pods were obtained 19 days after the second sampling (instead of seed and straw specimens), as the crop died due to heavy disease attack prior to reaching bean maturity. Untreated samples were taken at every time point. All specimens were continuously stored frozen until analysis.

Table 6.3/60 Target application rates and timings for dried beans

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
1999	7	1	F	BAS 351 32 H (SL)	Bentazone	1.552	300	BBCH 12

2. Description of analytical procedures

All samples were analysed for residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone according to BASF Method No. 438/1 with a limit of quantitation of 0.02 mg/kg for each analyte.

Bentazone and its metabolites were extracted from plant matrices with aqueous methanol. After purification by an iso-octane liquid-liquid partition, the two relevant metabolites which are present as glucosides were hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic plant constituents, a reversed phase C_{18}-column clean-up was performed. The analytes were then methylated with diazomethane and their derivatives were purified using a silica gel-column. The final determination of the residues of bentazone and its hydroxy metabolites was performed by GC-MS.

II. RESULTS AND DISCUSSION

The residue ranges are presented in Table 6.3/61. Details can be found in Table 6.3/62 and Table 6.3/63.
The analytical results indicate that the tested use of BAS 351 32 H did not produce quantifiable residues of bentazone and 6-OH-bentazone in specimens of commercially mature bean seeds irrespective of the cultivation type (pole or bush beans). 8-OH-bentazone was detected in one out of the seven seeds specimens just above the limit of quantitation (0.023 mg/kg).
The shoot specimens collected 12-31 days after the single application showed bentazone residues ranging from <0.02-0.04 mg/kg, as well as 6-OH-bentazone ranging from 0.044-4.31 mg/kg, and 8-OH-bentazone ranging from 0.030-0.356 mg/kg.
Bean pods collected 48-77 days after the application had no quantifiable residue of bentazone, 6-OH-bentazone, and 8-OH-bentazone.
One out of the seven straw specimens obtained at harvest showed a residue of bentazone at 0.083 mg/kg.
In four out of the seven specimens 6-OH-bentazone up to 0.229 mg/kg was detectable and 8-OH-bentazone was found in one of the seven specimens at 0.03 mg/kg.
No residues above the LOQ of 0.02 mg/kg of bentazone and metabolites were detected in any of the untreated control specimens.

Table 6.3/61 Summary of residues in dried beans

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	1999	12-31	22-32	shoots	<0.02-0.040	0.663-4.31	0.150-0.356	0.78-4.32
		48-68	79	pods	<0.02	<0.02	<0.02	<0.06
		82-101	**88-89**	**seeds**	**<0.02**	**<0.02**	**<0.02-0.023**	**<0.06-0.06**
		82-101	88-89	straw	<0.02	0.036-0.229	<0.02-0.030	0.07-0.26
EU South	1999	17-24	29-31	shoots	<0.02-0.021	0.044-1.01	0.030-0.092	0.10-1.05
		52-77	79	pods	<0.02	<0.02	<0.02	<0.06
		66-107	**79-89**	**seeds**	**<0.02**	**<0.02**	**<0.02**	**<0.06**
		66-107	79-89	straw/pods*	<0.02-0.083	<0.02-0.046	<0.02	<0.06-0.12

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
* without seed
Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Total bentazone residues in dry beans (seeds) were at or below the LOQ of 0.06 mg/kg at 66-107 days after the application (BBCH 79-89).

Table 6.3/62 Residues of bentazone in dried beans after one application of BAS 351 32 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	BSF 608/002850	Bush bean	The Netherlands	BAS 351 32 H: 1 x 1.552	12	12	shoots	0.040	4.31	0.251	4.32	**Method:** No. 438/1; LOQ = 0.02 mg/kg				
Doc ID:	2001/1000926					48	pods	<0.02	<0.02	<0.02	<0.06	Bentazone				
Trial No.	BSF/608-1					82	seeds	<0.02	<0.02	<0.02	<u>0.06</u>	shoots	0.02-2.0	3	71.1	26
GLP:	yes					82	straw	<0.02	0.229	0.030	0.26	pods	0.02-0.2	2	97.8	N/A
Year:	1999											seeds	0.02-0.2	2	88.6	N/A
Study code:	BSF 608/002850	Bush bean	France (North)	BAS 351 32 H: 1 x 1.552	12	31	shoots	<0.02	0.663	0.150	0.78	straw	0.02-0.2	2	82.6	N/A
Doc ID:	2001/1000926					57	pods	<0.02	<0.02	<0.02	<0.06	6-OH-bentazone				
Trial No.	BSF/608-2					101	seeds	<0.02	<0.02	<0.02	<u>0.06</u>	shoots	0.02-2.0	3	75.3	13
GLP:	yes					101	straw	<0.02	0.036	<0.02	0.07	pods	0.02-0.2	2	79.5	N/A
Year:	1999											seeds	0.02-0.2	2	88.0	N/A
Study code:	BSF 608/002850	Pole bean	France (North)	BAS 351 32 H: 1 x 1.552	13	14	shoots	0.032	3.62	0.356	3.76	straw	0.02-0.2	2	93.8	N/A
Doc ID:	2001/1000926					68	pods	<0.02	<0.02	<0.02	<0.06	8-OH-bentazone				
Trial No.	BSF/608-3					89	seeds	<0.02	<0.02	0.023	<u>0.06</u>	shoots	0.02-2.0	3	66.1	15
GLP:	yes					89	straw	<0.02	0.042	<0.02	0.08	pods	0.02-0.2	2	94.8	N/A
Year:	1999											seeds	0.02-0.2	2	91.0	N/A
												straw	0.02-0.2	2	69.0	N/A

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.02 + 0.02 + 0.02 = 0.06; 0.02 + <0.02 + <0.02 = 0.06; <0.02 + <0.02 + <0.02 = <0.06
6) at application
_ underlined values were used for MRL calculation
N/A not applicable

Table 6.3/63 Residues of bentazone in dried beans after one application of BAS 351 32 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	BSF 608/002850	Bush bean	France (South)	BAS 351 32 H: 1 x 1.552	12	17	shoots	0.021	1.01	0.092	1.05	**Method:** No. 438/1; LOQ = 0.02 mg/kg				
Doc ID:	2001/1000926					52	pods	<0.02	<0.02	<0.02	<0.06	Bentazone				
Trial No.	BSF/608-4					66	seeds	<0.02	<0.02	<0.02	<0.06	shoots	0.02-2.0	3	71.1	26
GLP:	yes					66	straw	<0.02	<0.02	<0.02	<0.06	pods	0.02-0.2	2	97.8	N/A
Year:	1999											seeds	0.02-0.2	2	88.6	N/A
Study code:	BSF 608/002850	Pole bean	France (South)	BAS 351 32 H: 1 x 1.552	12	18	shoots	<0.02	0.860	0.072	0.89	straw	0.02-0.2	2	82.6	N/A
Doc ID:	2001/1000926					59	pods	<0.02	<0.02	<0.02	<0.06	6-OH-bentazone				
Trial No.	BSF/608-5					105	seeds	<0.02	<0.02	<0.02	<0.06	shoots	0.02-2.0	3	75.3	13
GLP:	yes					105	straw	<0.02	0.046	<0.02	0.08	pods	0.02-0.2	2	79.5	N/A
Year:	1999											seeds	0.02-0.2	2	88.0	N/A
Study code:	BSF 608/002850	Bush bean	Spain	BAS 351 32 H: 1 x 1.552	12	24	shoots	<0.02	0.044	0.037	0.10	straw	0.02-0.2	2	93.8	N/A
Doc ID:	2001/1000926					77	pods	<0.02	<0.02	<0.02	<0.06	8-OH-bentazone				
Trial No.	BSF/608-6					107	seeds	<0.02	<0.02	<0.02	<0.06	shoots	0.02-2.0	3	66.1	15
GLP:	yes					107	straw	0.083	<0.02	<0.02	0.12	pods	0.02-0.2	2	94.8	N/A
Year:	1999											seeds	0.02-0.2	2	91.0	N/A

Table 6.3/63 Residues of bentazone in dried beans after one application of BAS 351 32 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	BSF 608/002850	Pole bean	Spain	BAS 351 32 H: 1 x 1.552	12-13	24	shoots	0.021	0.094	0.030	0.14	straw	0.02-0.2	2	69.0	N/A
Doc ID:	2001/1000926					66	pods	<0.02	<0.02	<0.02	<0.06					
Trial No.	BSF/608-7					85	seeds	<0.02	<0.02	<0.02	<u>≤0.06</u>					
GLP:	yes					85	pods*	<0.02	<0.02	<0.02	<0.06					
Year:	1999															

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.02 + 0.02 + 0.02 = 0.06; 0.02 + <0.02 + <0.02 = 0.06; <0.02 + <0.02 + <0.02 = <0.06
6) at application
* without seed
_ underlined values were used for MRL calculation
N/A not applicable

Report:	II A 6.3.6/4 Stroemel C. et al. 2000(b) Study on the residue behavior of Bentazone in beans (PHSVN) after treatment with BAS 351 32 H under field conditions in Germany, 1999 BASF DocID 2000/1014883
Guidelines:	BBA IV 3-3; IVA Guidelines for Residue Studies Sections IA and IB 2nd edition 1992
GLP:	Yes (laboratory certified by Land Brandenburg Ministerium fuer Landwirtschaft, Umweltschutz und Raumordnung, Potsdam, Germany)

Executive Summary

During the 1999 growing season, two field trials were conducted in Northern Europe to determine the residue levels of bentazone, 6-OH-bentazone and 8-OH-bentazone in bean matrices. The herbicidal test substance BAS 351 32 H (480 g/L bentazone, SL) was applied once at an application rate of about 1.536 kg a.s./ha. The applications were performed after the second leaf was unfolded. For the analysis, plant samples without roots were collected 20-25 days after application. Pods with beans were taken 47-51 days thereafter. Finally, dry beans and straw with pods were sampled at normal crop maturity which was 89-98 days after application. In the following, only the information on the dry beans is given. Details on fresh beans are presented in chapter 6.3.12.
All samples were analysed for residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone according to BASF Method No. 438/1 with a limit of quantitation of 0.02 mg/kg for each analyte. Total bentazone residues in dry beans were below the LOQ of 0.06 mg/kg at 89-98 DALA (BBCH 87-89). Therefore, the residue values were used for MRL determination (see chapter 6.7) even though an exaggerated application rate compared to the critical GAP was used.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 32 H (SL)
Lot/Batch #:	98-1; bentazone 480 g/L (nominal)
Purity:	Not reported
CAS#:	25057-89-0
Development code:	Not reported
Spiking levels:	0.02-2.0 mg/kg

2. Test Commodity:

Crop:	Bean (dry)
Type:	Pulses
Variety:	Berggold, Goldtime
Botanical name:	*Phaseolus vulgaris*
Crop parts(s) or processed commodity:	Plant without root, beans with pods, dry beans, straw with pods
Sample size:	Not reported

B. STUDY DESIGN

1. Test procedure

During the 1999 growing season, two field trials were conducted in representative bean growing areas in Germany to determine the residue levels of bentazone, 6-OH-bentazone and 8-OH-bentazone in bean matrices.

The herbicidal test substance BAS 351 32 H (485 g/L bentazone, SL) was applied once at an application rate of about 3.2 L/ha, equivalent to 1.536 kg a.s./ha, and a spray volume of about 300 L/ha. The applications were performed after the second leaf was unfolded.

For the analysis, plant samples without roots were collected 20-25 days after application. Pods with beans were taken 47-51 days thereafter. Finally, dry beans and straw with pods were sampled at normal crop maturity which was 89-98 days after application.

Untreated samples were taken at every time point. All specimens were stored frozen until analysis.

Table 6.3/64 Target application rates and timings for dried beans

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
1999	2	1	F	BAS 351 32 H (SL)	Bentazone	1.536	300	BBCH 12

2. Description of analytical procedures

All samples were analysed for residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone according to BASF Method No. 438/1 with a limit of quantitation of 0.02 mg/kg for each analyte.

Bentazone and its metabolites were extracted from plant matrices with aqueous methanol. After purification by an iso-octane liquid-liquid partition, the two relevant metabolites which are present as glucosides were hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic plant constituents, a reversed phase C_{18}-column clean-up was performed. The analytes were then methylated with diazomethane and their derivatives were purified using a silica gel-column. The final determination of the residues of bentazone and its hydroxy metabolites was performed by GC-MS.

II. RESULTS AND DISCUSSION

The residue ranges are presented in Table 6.3/65. Details can be found in Table 6.3/66.
In plants without roots, bentazone residues ranged from <0.02-0.026 mg/kg, while residues of 6-OH-bentazone and 8-OH-bentazone were 0.804-0.941 and 0.168-0.212 mg/kg, respectively. Total residues accounted for 0.94-1.10 mg/kg.
In dry bean, no residue of any compound above the LOQ (0.02 mg/kg) was found.
In straw, bentazone residues ranged from <0.02-0.078 mg/kg, while residues of 6-OH-bentazone and 8-OH-bentazone were 0.045-0.046 and <0.02-0.022 mg/kg, respectively. Total residues accounted for 0.08-0.14 mg/kg.
No residues above the LOQ of 0.02 mg/kg of bentazone and metabolites were detected in any of the untreated control specimens.

Table 6.3/65 Summary of residues in dried beans

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	1999	20-25	30-51	plant[5]	<0.02-0.026	0.804-0.941	0.168-0.212	0.94-1.10
		47-51	79	beans*	<0.02	<0.02	<0.02	<0.06
		89-98	**87-89**	**dry beans**	**<0.02**	**<0.02**	**<0.02**	**<0.06**
		89-98	87-89	straw*	<0.02-0.078	0.045-0.046	<0.02-0.022	0.08-0.14

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) without roots
* with pods
Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Total bentazone residues in dry beans were below the LOQ of 0.06 mg/kg at 89-98 DALA (BBCH 87-89).

Table 6.3/66 Residues of bentazone in fresh and dried beans after one application of BAS 351 32 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	AC/BASF/01/99	Bean	Germany	BAS 351 32 H: 1 x 1.536	12	20	plant[5]	0.026	0.804	0.168	0.94	**Method:** No. 438/1; LOQ = 0.02 mg/kg				
Doc ID:	2000/1014883					47	beans*	<0.02	<0.02	<0.02	<0.06	Bentazone				
Trial No.	AC/99/29					89	dry beans	<0.02	<0.02	<0.02	<u>≤0.06</u>	plant[5]	0.02-2.0	2	94.5	N/A
GLP:	yes					89	straw*	<0.02	0.046	<0.02	0.08	beans*	0.02-0.2	2	103	N/A
Year:	1999											dry beans	0.02-0.2	2	101	N/A
Study code:	AC/BASF/01/99	Bean	Germany	BAS 351 32 H: 1 x 1.536	12	25	plant[5]	<0.02	0.941	0.212	1.10	straw*	0.02-0.2	2	104	N/A
Doc ID:	2000/1014883					51	beans*	<0.02	<0.02	<0.02	<0.06	6-OH-bentazone				
Trial No.	AC/99/30					98	dry beans	<0.02	<0.02	<0.02	<u>≤0.06</u>	plant[5]	0.02-2.0	2	94.6	N/A
GLP:	yes					98	straw*	0.078	0.045	0.022	0.14	beans*	0.02-0.2	2	84.8	N/A
Year:	1999											dry beans	0.02-0.2	2	85.0	N/A
												straw*	0.02-0.2	2	103	N/A
												8-OH-bentazone				
												plant[5]	0.02-2.0	2	99.7	N/A
												beans*	0.02-0.2	2	94.7	N/A
												dry beans	0.02-0.2	2	97.9	N/A
												straw*	0.02-0.2	2	102	N/A

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.02 + 0.02 + 0.02 = 0.06; 0.02 + <0.02 + <0.02 = 0.06; <0.02 + <0.02 + <0.02 = <0.06
5) without roots
6) at application
* with pods
_ underlined values were used for MRL calculation
N/A not applicable

Report:	II A 6.3.6/5 Oxspring S. 2008(i) Study on the residue behaviour of Bentazone in dried beans after treatment with BAS 351 45 H under field conditions in Northern and Southern Europe during 2007 BASF DocID 2008/1049971
Guidelines:	None
GLP:	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

During the 2007 growing season, four field trials on beans were conducted in Northern and Southern Europe to determine the residue level of bentazone (BAS 351 45 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC). BAS 351 45 H, an SG formulation of bentazone (87% w/w), was foliar applied once at a rate equivalent to 0.957 kg a.s./ha to dried beans at 28 days before expected harvest. Specimens of whole plant (without roots) were collected immediately after the application had dried (0 DALA) from each plot as well as seeds that had been mechanically harvested and seeds and empty pods that were manually harvested 28 days after application.

Specimens were analysed using SOP-0297, based on BASF Method No. 438/2, to quantify the residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone. The limit of quantitation (LOQ) of the method was 0.01 mg/kg for each analyte. The values detected for mechanically and manually harvested seed specimens were at a similar level. Total bentazone residues in mechanically and manually harvested dried seed specimens were 0.09-0.37 mg/kg and 0.05-0.31 mg/kg, respectively, at 28 days after the application.

The results from this study were not used for MRL determination since the application was performed rather close to harvest and therefore the resulting residues are comparatively high. However, the results from this study show that the values detected for mechanically and manually harvested seed specimens were at a similar level.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 351 45 H (SG)
Lot/Batch #: 2002-1, 87%
Purity: Not reported
CAS#: 25057-89-0
Development code: Not reported
Spiking levels: 0.01-5.0 mg/kg

2. Test Commodity:

Crop: Bean (dry)
Type: Pulses
Variety: Cinco, Linex, Melodie
Botanical name: *Phaseolus vulgaris*
Crop parts(s) or processed commodity: Whole plants without roots, seed, pods without seeds
Sample size: 1 kg / 12 units (nominal)

B. STUDY DESIGN

1. Test procedure

During the 2007 growing season, four field trials were conducted in representative dried bean growing areas in France and the United Kingdom to determine the residue level of bentazone (BAS 351 45 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).

BAS 351 45 H, an SG formulation of bentazone (87% w/w), was foliar applied once at a rate of 1.1 kg of formulated product /ha, equivalent to 0.957 kg a.s./ha, to dried beans at 28 days before expected harvest. The nominal spray volume used was 200 L/ha.

Specimens of whole plant (without roots) were collected immediately after the application had dried (0 DALA) from each plot as well as seeds that had been mechanically harvested and seeds and empty pods that were manually harvested 28 days after application.

Untreated control specimens were taken at every time point. All specimens were stored frozen (≤-18°C) until analysis. The maximum storage interval from harvest until analysis was 227 days.

Table 6.3/67 Target application rates and timings for dried beans

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2007	4	1	F	BAS 351 45 H (SG)	Bentazone	0.957	200	28 days before harvest

2. Description of analytical procedures

Specimens were analysed using SOP-0297, based on BASF Method No. 438/2, to quantify the residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone. Residues were extracted from plant matrices using a mixture of methanol and water. The two metabolites that were present as glucosides were hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$ precipitation step to remove acidic plant constituents, a reversed phase C_{18}-column clean-up was performed. The final determination of the residues of bentazone and its hydroxyl metabolites was performed by LC-MS/MS. The limit of quantitation (LOQ) of the method was 0.01 mg/kg for each analyte.

II. RESULTS AND DISCUSSION

The residue ranges are presented in Table 6.3/68. Details can be found in Table 6.3/69 and Table 6.3/70.

Directly after the application residues of bentazone and its metabolites ranged from 3.06 to 3.46 mg/kg in whole plants (without roots). Bentazone accounted for 1.9 to 3.0 mg/kg of the total residue, while 6-OH-bentazone and 8-OH-bentazone could be detected at levels of between 0.4 and 0.7 mg/kg and 0.09 and 0.6 mg/kg respectively.

At 28 days after application residues decreased significantly. The values detected for mechanically and manually harvested seed specimens were at a similar level. Residues detected in mechanically harvested seed were between 0.04 and 0.08 mg/kg for bentazone, 0.03 and 0.3 mg/kg for 6-OH-bentazone and <0.01 mg/kg for 8-OH-bentazone. Manually harvested seed specimens showed residues ranging from <0.01 to 0.02 mg/kg for bentazone, 0.01 to 0.3 mg/kg for 6-OH-bentazone and <0.01 to 0.2 mg/kg for 8-OH-bentazone. The residue levels for pods without seeds sampled at 28 days after application were 0.01 to 1.4 mg/kg for bentazone, 0.8 to 3.2 mg/kg and 0.05 to 0.4 mg/kg for 6-OH-bentazone and 8-OH-bentazone respectively.

Table 6.3/68 Summary of residues in dried beans

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	2007	0	79-80	whole plants[5]	2.40-3.00	0.40	0.09-0.30	3.06-3.46
		28	87-89	pods*	0.80-1.40	0.80-3.20	0.05-0.06	2.21-3.85
		28	**87-89**	**seed****	**0.04-0.08**	**0.30**	**<0.01**	**0.33-0.37**
		28	**87-89**	**seed*****	**<0.01-0.02**	**0.30**	**<0.01**	**0.30-0.31**
EU South	2007	0	76	whole plants[5]	1.90-2.80	0.50-0.70	0.10-0.60	3.12-3.36
		28	89	pods*	0.01-0.02	1.20	0.30-0.40	1.42-1.53
		28	**89**	**seed****	**0.05-0.08**	**0.03-0.04**	**<0.01**	**0.09-0.13**
		28	**89**	**seed*****	**<0.01**	**0.01-0.03**	**<0.01-0.20**	**0.05-0.21**

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) without roots

* without seeds, manually harvested
** mechanically harvested
*** manually harvested
Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

The values detected for mechanically and manually harvested seed specimens were at a similar level. Total bentazone residues in mechanically and manually harvested dried seed specimens were 0.09-0.37 mg/kg and 0.05-0.31 mg/kg, respectively, at 28 days after the application.

Table 6.3/69 Residues of bentazone in dried beans after one application of BAS 351 45 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	303256	Dried bean	United Kingdom	BAS 351 45 H: 1 x 0.957	80	0	plant[5]	3.00	0.40	0.09	3.46	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2008/1049971					28	pods*	0.80	3.20	0.05	3.85	Bentazone				
Trial No.	L070805					28	seed**	0.04	0.30	<0.01	0.33	all matrices	0.01-5.0	7	95	15
GLP:	yes					28	seed***	<0.01	0.30	<0.01	0.30	6-OH-bentazone				
Year:	2007											all matrices	0.01-5.0	7	80	18
Study code:	303256	Dried bean	France (North)	BAS 351 45 H: 1 x 0.957	79-80	0	plant[5]	2.40	0.40	0.30	3.06	8-OH-bentazone				
Doc ID:	2008/1049971					28	pods*	1.40	0.80	0.06	2.21	all matrices	0.01-5.0	7	79	13
Trial No.	L070920					28	seed**	0.08	0.30	<0.01	0.37					
GLP:	yes					28	seed***	0.02	0.30	<0.01	0.31					
Year:	2007															

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) at application
* without seeds, manually harvested
** mechanically harvested
*** manually harvested

Table 6.3/70 Residues of bentazone in dried beans after one application of BAS 351 45 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	303256	Dried bean	France (South)	BAS 351 45 H: 1 x 0.957	76	0	plant[5]	2.80	0.50	0.10	3.36	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2008/1049971					28	pods*	0.02	1.20	0.40	1.53	Bentazone				
Trial No.	L070806					28	seed**	0.08	0.04	<0.01	0.13	all matrices	0.01-5.0	7	95	15
GLP:	yes					28	seed***	<0.01	0.01	0.20	0.21	6-OH-bentazone				
Year:	2007											all matrices	0.01-5.0	7	80	18
Study code:	303256	Dried bean	France (South)	BAS 351 45 H: 1 x 0.957	76	0	plant[5]	1.90	0.70	0.60	3.12	8-OH-bentazone				
Doc ID:	2008/1049971					28	pods*	0.01	1.20	0.30	1.42	all matrices	0.01-5.0	7	79	13
Trial No.	L070807					28	seed**	0.05	0.03	<0.01	0.09					
GLP:	yes					28	seed***	<0.01	0.03	<0.01	0.05					
Year:	2007															

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without pods
6) at application
* with seeds
** mechanically harvested
*** manually harvested

Peas

Report:	II A 6.3.6/6 Oxspring S. 2008(g) Study on the residue behaviour of Bentazone in fresh and dried peas after treatment with BAS 351 45 H under field conditions in Northern and Southern Europe during 2007 BASF DocID 2008/1049972
Guidelines:	None
GLP:	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

During the 2007 growing season, four field trials were conducted in representative fresh and dried pea growing areas in France (dried peas) and the United Kingdom (fresh peas) to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone (BH 351-6-OH) and 8-OH-bentazone (BH 351-8-OH) in or on raw agricultural commodities (RAC). BAS 351 45 H, an SG formulation of bentazone (87% w/w), was foliar applied once at a rate equivalent to 0.957 kg a.s./ha to fresh and dried peas at 28 days before expected harvest. Specimens of whole plant (without roots or pods) and whole pods were collected immediately after the application had dried (0 DAA) from each plot, as well as seeds that had been mechanically harvested and seeds and whole pods that were manually harvested 28 days after application. In the following, only the information on the dried pea trials is given. Details on fresh peas are presented in chapter 6.3.12.
Residues were determined by means of BASF Method No. 438/2; the limit of quantitation was 0.01 mg/kg for all analytes. In dried peas, at 28 days after application, total bentazone residues were 0.15-0.51 mg/kg in seeds and 0.51-2.80 mg/kg in pods. The metabolite 6-OH-bentazone contributed most to the total residue in seed. Residues were in the same range in the mechanically and manually harvested seed samples.
The results from this study were not used for MRL determination since the application was performed rather close to harvest and therefore the resulting residues are quite high. However, the results from this study show that the values detected for mechanically and manually harvested seed specimens were at a similar level.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 351 45 H
Lot/Batch #: 2002-1, 87% bentazone, nominal
Purity: Not reported
CAS#: 25057-89-0
Development code: Not reported
Spiking levels: 0.01-5.0 mg/kg

2. Test Commodity:

Crop: Pea (dry)
Type: Pulses
Variety: Lucy, Ideal
Botanical name: *Pisum sativum*
Crop parts(s) or processed commodity: Whole plant without root or pods, whole pods (mechanically harvested), seed (mechanically and manually harvested)
Sample size: Min. 1 kg / 12-24 units (nominal)

B. STUDY DESIGN

1. Test procedure

During the 2007 growing season, four field trials were conducted in representative fresh and dried pea growing areas in France (dried peas) and the UK (fresh peas) to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone (BH 351-6-OH) and 8-OH-bentazone (BH 351-8-OH) in or on raw agricultural commodities (RAC).

BAS 351 45 H, an SG formulation of bentazone (87% w/w), was foliar applied once at a rate equivalent to 0.957 kg a.s./ha to fresh and dried peas at 28 days before expected harvest. The nominal spray volume used was 200 L/ha.

Specimens of whole plant (without roots or pods) and whole pods were collected immediately after the application had dried (0 DALA) from each plot, as well as seeds that had been mechanically harvested and seeds and whole pods that were manually harvested 28 days after application. However, at Trials AF/12152/BAI1 and 2 (L070800 and L070801) the fresh peas were not mature enough to sample whole pods at the 0 DALA sampling timing.

In the following, only the information on the dry pea trials is given. Details on fresh peas are presented in chapter 6.3.12.

Table 6.3/71 Target application rates and timings for dried peas

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2007	2	1	F	BAS 351 45 H (SG)	BAS 351 H	0.957	200	28±1 days before harvest

2. Description of analytical procedures

All samples were analysed according to BASF Method No. 438/2. Bentazone, 6-OH-bentazone and 8-OH-bentazone are extracted from plant material using a mixture of methanol and water. The two metabolites that were present as glucosides were hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$ precipitation step to remove acidic plant constituents, a reversed phase C_{18} column clean-up was performed or the extract was filtered and one aliquot taken, evaporated to dryness and dissolved in a methanol/water solution. The final determination of the residues of bentazone and its hydroxyl metabolites was performed by LC-MS/MS. The limit of quantitation was 0.01 mg/kg for all substances.

II. RESULTS AND DISCUSSION

The residue ranges in peas determined at PHIs between 0 and 28 days are presented in Table 6.3/72. Details can be found in Table 6.3/73.

Directly after application residues of bentazone and its metabolites were 26.22 to 26.27 mg/kg in whole plants without root or pods. Bentazone accounted for 24.34 to 25.11 mg/kg of the total residue, while 6-OH-bentazone and 8-OH-bentazone could be detected at levels of 0.92 to 1.62 mg/kg and 0.32 to 0.38 mg/kg respectively. In whole pod specimens lower residue levels were detected at 1.96 to 6.26 mg/kg for bentazone, 0.13 to 0.20 mg/kg for 6-OH-bentazone and 0.03 mg/kg for 8-OH-bentazone.

At 28 days after application residues decreased significantly. The values detected for mechanically and manually harvested seed specimens were at a similar level; in mechanically harvested seed between 0.04 and 0.11 mg/kg for bentazone, 0.31 and 0.34 mg/kg for 6-OH-bentazone and <0.01 mg/kg for 8-OH-bentazone. Manually harvested seed specimens showed residues of 0.01 to 0.20 mg/kg for bentazone, 0.14 to 0.32 mg/kg for 6-OH-bentazone and <0.01 mg/kg for 8-OH-bentazone. The residue levels for whole pods sampled 28 days after application were 0.21 to 2.28 mg/kg for bentazone and 0.31 to 0.54 mg/kg and <0.01 to 0.02 mg/kg for 6-OH-bentazone and 8-OH-bentazone, respectively.

Table 6.3/72 Summary of residues in dried peas

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	2007	0	69-73	whole plant[5]	24.34-25.11	0.92-1.62	0.32-0.38	26.22-26.27
		0	69-73	whole pods	1.96-6.26	0.13-0.20	0.03	2.11-6.48
		28	**89**	**seed***	**0.04-0.11**	**0.31-0.34**	**<0.01**	**0.37-0.41**
		28	**89**	**seed****	**0.01-0.20**	**0.14-0.32**	**<0.01**	**0.15-0.51**
		28	89	whole pods**	0.21-2.28	0.31-0.54	<0.01-0.02	0.51-2.80

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) without root or pods

* mechanically harvested
** manually harvested
Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

At 28 days after application, total bentazone residues were 0.15-0.51 mg/kg in seeds and 0.51-2.80 mg/kg in pods. The metabolite 6-OH-bentazone contributed most to the total residue. Residues were in the same range in the mechanically and manually harvested seed samples.

Table 6.3/73 Residues of bentazone in dried peas after one application of BAS 351 45 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	303257	Dry pea	France (South)	BAS 351 45 H: 1 x 0.957	69-70	0	plant[5]	25.11	0.92	0.32	26.27	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2008/1049972					0	wh. pod	6.26	0.20	0.03	6.48	Bentazone				
Trial No.	L070802					28	seed*	0.11	0.31	<0.01	0.41	seeds/pods	0.01-1.0	7	88	14
GLP:	yes					28	seed**	0.20	0.32	<0.01	0.51	w. plant[5]	0.01-5.0	6	86	10
Year:	2007					28	wh. pod**	2.28	0.54	0.02	2.80	6-OH-bentazone				
Study code:	303257	Dry pea	France (South)	BAS 351 45 H: 1 x 0.957	69-73	0	plant[5]	24.34	1.62	0.38	26.22	seeds/pods	0.01-5.0	9	92	14
Doc ID:	2008/1049972					0	wh. pod	1.96	0.13	0.03	2.11	w. plant[5]	0.01-1.0	4	84	5
Trial No.	L070803					28	seed*	0.04	0.34	<0.01	0.37	8-OH-bentazone				
GLP:	yes					28	seed**	0.01	0.14	<0.01	0.15	seeds/pods	0.01-1.0	7	83	7
Year:	2007					28	wh. pod**	0.21	0.31	<0.01	0.51	w. plant[5]	0.01-5.0	6	82	6

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots or pods
6) at application
* mechanically harvested
** manually harvested

6.3.7 Oilseeds

Soybean

Table 6.3/74 GAP for the use of BAS 351 H in/on soybeans

Crop	Maximum Applied Dose	Water Volume	PHI	Application Method	Application Timing
Soybean	1 x 0.96 kg BAS 351 H/ha	200-400 L/ha	F	spray application	BBCH 11-13

PHI = pre-harvest interval

Table 6.3/75 GAP information of residue trials conducted in soybeans between 1999 and 2009

Region	Country	Formulation	Application[0]				DALA[1]
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
EU North	Germany (1 trial)	BAS 351 45 H, SG	spray application	0.957	0.479	1	0 30 79
	France (1 trial)	BAS 762 01 H, SL	spray application	0.914	0.450	1	90
	France (1 trial)	BAS 762 01 H, SL	spray application	0.924	0.450		90
EU South	Greece (1 trial)	BAS 351 45 H, SG	spray application	0.957	0.479	1	0 29-31 68-93
	Italy (1 trial)						
	Spain (1 trial)						
	France (1 trial)						
	France (2 trials)	BAS 762 01 H, SL	spray application	0.906-0.987	0.451	1	90-104
	Italy (2 trials)			0.900-0.937	0.225		85-88
	France (2 trials)	BAS 762 01 H, SL	spray application	0.866-0.960	0.449-0.451	1	90-104
	Italy (2 trials)			0.900-0.923	0.225		85-88

0) actual application rates varied by 10% at most
1) days after last application

Report:	II A 6.3.7/1 Schroth E., Martin T. 2010(j) Study on the residue behavior of BAS 351 H (Bentazone) in soybean after the application of BAS 351 45 H under field conditions in Germany, Greece, France (South), Italy and Spain, 2009 BASF DocID 2010/1164275
Guidelines:	EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8); EEC 1607/VI/97 rev. 2 10.06.1999; EEC 7029/VI/95 rev. 5; EEC 7525/VI/95 rev. 7
GLP:	Yes (laboratory certified by ENAC, Entidad Nacional de Acreditación, Madrid, Spain)

Executive Summary

During the 2009 growing season 5 trials in soybean were conducted in different representative growing areas in Germany, Greece, France (South), Italy and Spain to determine the residue level of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC). BAS 351 45 H (87% bentazone, SG) was applied once at a rate equivalent to 0.957 kg a.s./ha of bentazone and the spray volume used was 200 L/ha. The application was done at growth stage BBCH 55. Specimens of whole plant without roots were collected at the day of the application. Specimens of rest of plant (no roots) and seed (in case of immaturity pods with seeds were sampled instead of seeds) were collected at 30±1 days after the application. Additionally, specimens of rest of plant (no roots) and seeds had to be taken at crop stage 89 BBCH (harvest) if this stage was not reached before.
The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. Total bentazone residues in seed specimens at BBCH 89 were <0.03 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 45 H (SG)
Lot/Batch #:	2002-1, 87% bentazone, nominal
Purity:	Not reported
CAS#:	25057-89-0
Development code:	Not reported
Spiking levels:	0.01-80 mg/kg

2. Test Commodity:

Crop:	Soybean
Type:	Oilseeds
Variety:	Merlin, Nikko, Blancas, CV Condor, Samera
Botanical name:	*Glycine max.*
Crop parts(s) or processed commodity:	Whole plant no roots, rest of plant (no roots), pods with seeds, seed
Sample size:	1.0-2.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2009 growing season 5 trials in soybean were conducted in different representative growing areas in Germany, Greece, France (South), Italy and Spain to determine the residue level of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC). BAS 351 45 H (87% bentazone, SG) was applied once at a rate equivalent to 0.957 kg a.s./ha of bentazone and the spray volume used was 200 L/ha. The application was done at growth stage BBCH 55. Specimens of whole plant without roots were collected at the day of the application. Specimens of rest of plant (no roots) and seed (in case of immaturity pods with seeds were sampled instead of seeds) were collected at 30±1 days after the application. Additionally, specimens of rest of plant (no roots) and seeds had to be taken at crop stage 89 BBCH (harvest) if this stage was not reached before.

Table 6.3/76 Target application rates and timings for soybeans

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2009	5	1	F	BAS 351 45 H (SG)	BAS 351 H	0.957	200	BBCH 55

2. Description of analytical procedures

The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg.

Samples were extracted with aqueous methanol, followed by iso-octane extraction and liquid/liquid partition of an aliquot. Hydrolysis was performed using enzymatic cleavage, and a $Ca(OH)_2$-precipitation step was included to remove acidic matrix constituents. Reversed phase C_{18}-SPE clean-up was performed before the final determination by liquid chromatography and tandem mass spectrometric detection (LC-MS/MS).

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3/77, detailed residue levels are shown in Table 6.3/78 and Table 6.3/79.
The bentazone residues in soybean whole plant no roots specimens taken directly after the application ranged between 25-95 mg/kg. At 29-31 DALA, the residues in rest of plant (no roots) ranged between <0.01-0.19 mg/kg and in pods with seeds ranged between 0.01-0.20 mg/kg. At 68-93 DALA (BBCH 89), residues in rest of plant (no roots) ranged between <0.01-0.12 mg/kg and residues in seed had declined below the LOQ (<0.01 mg/kg).
The 6-OH-bentazone residues in soybean whole plant no roots specimens taken directly after the application ranged between 0.33-1.7 mg/kg. At 29-31 DALA, residues in rest of plant (no roots) ranged between 0.47-2.4 mg/kg and in pods with seeds ranged between <0.01-0.02 mg/kg. At BBCH 89 (68-93 DALA) residues in rest of plant (no roots) ranged between 0.05-0.46 mg/kg and residues in seed had declined below the LOQ (<0.01 mg/kg).
The 8-OH-bentazone residues in soybean whole plant no roots specimens taken directly after the application ranged between 0.52-1.4 mg/kg. At 29-31 DALA, residues in rest of plant (no roots) ranged between 0.34-1.9 mg/kg and in pods with seeds ranged between <0.01-0.02 mg/kg. At BBCH 89 (68-93 DALA) residues in rest of plant (no roots) ranged between 0.04-0.39 mg/kg and residues in seed had declined below the LOQ (<0.01 mg/kg).
The total bentazone residues in soybean whole plant no roots specimens taken directly after the application ranged between 25.80-96.57 mg/kg. At 29-31 DALA, residues in rest of plant (no roots) ranged between 0.77-4.06 mg/kg and in pods with seeds ranged between 0.03-0.22 mg/kg. At BBCH 89 (68-93 DALA) residues in rest of plant (no roots) ranged between 0.10-0.82 mg/kg and residues in seed had declined below 0.03 mg/kg.
No residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone above the limit of quantitation (0.01 mg/kg) were found in the control specimens of this study.

Table 6.3/77 Summary of residues in soybeans

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	2009	0	55	whole plant[5]	25	0.33	0.52	25.80
		30	81	rest of plant[5]	0.03	2.4	1.9	4.06
		30	81	pods[6]	0.20	0.01	0.01	0.22
		79	89	rest of plant[5]	0.01	0.19	0.17	0.35
		79	**89**	**seeds**	**<0.01**	**<0.01**	**<0.01**	**<0.03**
EU South	2009	0	55	whole plant[5]	50-95	0.63-1.7	1.0-1.4	51.81-96.57
		29-31	75-77	rest of plant[5]	<0.01-0.19	0.47-2.0	0.34-1.5	0.77-3.20
		29-31	75-77	pods[6]	<0.01-0.06	<0.01-0.02	<0.01-0.02	0.03-0.08
		68-93	89	rest of plant[5]	<0.01-0.12	0.05-0.46	0.04-0.39	0.10-0.82
		68-93	**89**	**seeds**	**<0.01**	**<0.01**	**<0.01**	**<0.03**

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) no roots
6) pods with seeds

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Total bentazone residues in seed specimens at BBCH 89 were <0.03 mg/kg.

Table 6.3/78 Residues of bentazone in soybean after one application of BAS 351 45 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	370252	Soybean	Germany	BAS 351 45 H: 1 x 0.957	55	0	plant[5]	25	0.33	0.52	25.80	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2010/1164275					30	rest[7]	0.03	2.4	1.9	4.06	Bentazone				
Trial No.	L090383					30	pods[8]	0.20	0.01	0.01	0.22	plant[5]	0.01-80	3	106	3.0
GLP:	yes					79	rest[7]	0.01	0.19	0.17	0.35	pods[8]	0.01, 0.1	2	106	N/A
Year	2009					79	seeds	<0.01	<0.01	<0.01	<u>≤0.03</u>	seeds	0.01, 0.1	2	100	N/A
												6-OH-bentazone				
												plant[5]	0.01-10	3	102	7.3
												pods[8]	0.01, 0.1	2	98	N/A
												seeds	0.01, 0.1	2	93	N/A
												8-OH-bentazone				
												plant[5]	0.01-4	3	96	3.0
												pods[8]	0.01, 0.1	2	97	N/A
												seeds	0.01, 0.1	2	94	N/A

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
   for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) whole plant no roots
6) at application
7) rest of plant no roots
8) pods with seeds

_ underlined values were used for MRL calculation

N/A not applicable

Table 6.3/79 Residues of bentazone in soybean after one application of BAS 351 45 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	370252	Soybean	Greece	BAS 351 45 H: 1 x 0.957	55	0	plant[5]	54	1.7	1.4	56.90	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2010/1164275					30	rest[7]	<0.01	0.47	0.34	0.77	Bentazone				
Trial No.	L090384					30	pods[8]	0.05	<0.01	<0.01	0.07	plant[5]	0.01-80	3	106	3.0
GLP:	yes					93	rest[7]	<0.01	0.05	0.04	0.10	pods[8]	0.01, 0.1	2	106	N/A
Year	2009					93	seeds	<0.01	<0.01	<0.01	<0.03	seeds	0.01, 0.1	2	100	N/A
Study code:	370252	Soybean	Italy	BAS 351 45 H: 1 x 0.957	55	0	plant[5]	50	0.63	1.3	51.81	6-OH-bentazone				
Doc ID:	2010/1164275					29	rest[7]	0.01	2.0	1.4	3.20	plant[5]	0.01-10	3	102	7.3
Trial No.	L090385					29	pods[8]	0.06	<0.01	0.01	0.08	pods[8]	0.01, 0.1	2	98	N/A
GLP:	yes					80	rest[7]	<0.01	0.13	0.13	0.25	seeds	0.01, 0.1	2	93	N/A
Year	2009					80	seeds	<0.01	<0.01	<0.01	<0.03	8-OH-bentazone				
Study code:	370252	Soybean	Spain	BAS 351 45 H: 1 x 0.957	55	0	plant[5]	95	0.67	1.0	96.57	plant[5]	0.01-4	3	96	3.0
Doc ID:	2010/1164275					31	rest[7]	0.19	0.92	0.87	1.87	pods[8]	0.01, 0.1	2	97	N/A
Trial No.	L090386					31	pods[8]	0.01	<0.01	<0.01	0.03	seeds	0.01, 0.1	2	94	N/A
GLP:	yes					68	rest[7]	0.12	0.13	0.12	0.35					
Year	2009					68	seeds	<0.01	<0.01	<0.01	<0.03					
Study code:	370252	Soybean	France	BAS 351 45 H: 1 x 0.957	55	0	plant[5]	92	0.67	1.3	93.85					
Doc ID:	2010/1164275					30	rest[7]	0.03	1.8	1.5	3.13					
Trial No.	L090387					30	pods[8]	<0.01	0.02	0.02	0.05					
GLP:	yes					77	rest[7]	0.02	0.46	0.39	0.82					
Year	2009					77	seeds	<0.01	<0.01	<0.01	<0.03					

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03

5) whole plant no roots
6) at application
7) rest of plant no roots
8) pods with seeds
_ underlined values were used for MRL calculation
N/A not applicable

Report:	II A 6.3.7/2 Kreke N. 2009(j) Determination of residues at harvest of Imazamox and Bentazone in soy bean (RAC seed) following one treatment with a tankmix of BAS 762 01 H (22.4/480 g/L) and BAS 9047 0S (DASH HC) from one open field trial in Northern France in 2008 BASF DocID 2008/1034457
Guidelines:	EEC 96/68; EEC 7029/VI/95 rev. 5
GLP:	Yes (laboratory certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	II A 6.3.7/3 Kreke N. 2010(q) Amendment: BAS 762 01 H with adjuvant BAS 9047 0S (DASH HC) - Determination of residues at harvest of Imazamox and Bentazone in soy bean (RAC seed) following one treatment with the tankmix from one open field trial in Northern France, 2008 BASF DocID 2010/1155811
Guidelines:	EEC 96/68; EEC 7029/VI/95 rev. 5
GLP:	Yes (laboratory certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Executive Summary

During the 2008 growing season, one trial in soybean was conducted in a representative growing area in Northern France, to determine the residue level of bentazone, its metabolites 6-OH-bentazone and 8-OH-bentazone and imazamox with its metabolite CL 263384 in or on raw agricultural commodities (RAC). In the following only residue data from bentazone as well as of its metabolites 6-OH-bentazone and 8-OH-bentazone are presented.
BAS 762 01 H (480 g/L bentazone, SL) was applied once at a rate equivalent to 0.914 kg a.s./ha of bentazone together with an adjuvant BAS 9047 OS (DASH HC) and the spray volume used was 200 L/ha. The application was done at growth stage BBCH 13. Specimens of soybean samples (RAC seeds) were collected at harvest and analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. Total bentazone residues in seed specimens at BBCH 89 were below the LOQ of 0.03 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 762 01 H (SL); BAS 9047 0S (DASH HC); adjuvant (0.5% by volume of spray solution
Lot/Batch #:	402003, 480 g/L bentazone (nominal); 1071, BAS 9047 0S
Purity:	Not reported
CAS#:	25057-89-0
Development code:	Not reported
Spiking levels:	0.01-0.1 mg/kg

2. Test Commodity:

Crop:	Soybean
Type:	Oilseeds
Variety:	Essor
Botanical name:	*Glycine max.*
Crop parts(s) or processed commodity:	Seeds
Sample size:	min. 1.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2008 growing season, one trial in soybean was conducted in a representative growing area in Northern France, to determine the residue level of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
BAS 762 01 H (480 g/L bentazone, SL) was applied once at a rate equivalent to 0.914 kg a.s./ha of bentazone together with an adjuvant BAS 9047 OS (DASH HC) and the spray volume used was 200 L/ha. The application was done at growth stage BBCH 13. Specimens of soybean samples (RAC seeds) were collected at harvest (BBCH 89).

Table 6.3/80 Target application rates and timings for soybeans

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2008	1	1	F	BAS 762 01 H (SL)	BAS 351 H	0.900	100-400	BBCH 12-25

2. Description of analytical procedures

The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg.
Samples were extracted with aqueous methanol, followed by iso-octane extraction and liquid/liquid partition of an aliquot. Hydrolysis was performed using enzymatic cleavage, and a $Ca(OH)_2$-precipitation step was included to remove acidic matrix constituents. Reversed phase C_{18}-SPE clean-up was performed before the final determination by liquid chromatography and tandem mass spectrometric detection (LC-MS/MS).

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3/81, detailed residue levels are shown in Table 6.3/82.
Residues of bentazone, 6-OH-bentazone and 8-OH-bentazone could not be detected after 90 DALA (BBCH 89).

Table 6.3/81 Summary of residues in soybeans

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	2008	**90**	**89**	**seeds**	**<0.01**	**<0.01**	**<0.01**	**<0.03**

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Total bentazone residues in seed specimens at BBCH 89 were below the LOQ of 0.03 mg/kg.

Table 6.3/82 Residues of bentazone in soybean after one application of BAS 762 01 H with adjuvant BAS 9047 0S (DASH HC) in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	B89785	Soybean	France	BAS 762 01 H: 1 x 0.914	13	90	seeds	<0.01	<0.01	<0.01	<u>≤0.03</u>	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2008/1034457											Bentazone				
	2010/1155811											seeds	0.01, 0.1	2	96	N/A
Trial No.	A/NF/H/08/49											6-OH-bentazone				
GLP:	yes											seeds	0.01, 0.1	2	85	N/A
Year	2008											8-OH-bentazone				
												seeds	0.01, 0.1	2	84	N/A

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
6) at application
_ underlined values were used for MRL calculation
N/A not applicable

Report:	II A 6.3.7/4 Kreke N. 2009(i) BAS 762 01 H: Determination of residues at harvest of Imazamox and Bentazone in soy bean (RAC seed) following one treatment with BAS 762 01 H (22.4/480 g/L Imazamox/Bentazone) from one open field trial in Northern France in 2008 BASF DocID 2008/1034456
Guidelines:	EEC 96/68; EEC 7029/VI/95 rev. 5
GLP:	Yes (laboratory certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	II A 6.3.7/5 Kreke N. 2010(r) First amendment: Determination of residues at harvest of Imazamox and Bentazone in soy bean (RAC seed) following one treatment with BAS 762 01 H (22.4/480 g/L Imazamox/Bentazone) from one open field in Northern France, 2008 BASF DocID 2010/1155810
Guidelines:	EEC 96/68; EEC 7029/VI/95 rev. 5
GLP:	Yes (laboratory certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Executive Summary

During the 2008 growing season, one trial in soybean was conducted in a representative growing area in Northern France, to determine the residue level of bentazone, its metabolites 6-OH-bentazone and 8-OH-bentazone and imazamox with its metabolite CL 263384 in or on raw agricultural commodities (RAC). In the following only residue data from bentazone as well as of its metabolites 6-OH-bentazone and 8-OH-bentazone are presented.
BAS 762 01 H (480 g/L bentazone, SL) was applied once at a rate equivalent to 0.924 kg a.s./ha of bentazone and the spray volume used was 205 L/ha. The application was done at growth stage BBCH 13. Specimens of soybean samples (RAC seeds) were collected at harvest and analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. Total bentazone residues in seed specimens at BBCH 89 were below the LOQ of 0.03 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 762 01 H (SL)
Lot/Batch #:	402003, 480 g/L bentazone (nominal)
Purity:	Not reported
CAS#:	25057-89-0
Development code:	Not reported
Spiking levels:	0.01-0.1 mg/kg

2. Test Commodity:

Crop:	Soybean
Type:	Oilseeds
Variety:	Essor
Botanical name:	*Glycine max.*
Crop parts(s) or processed commodity:	Seeds
Sample size:	min. 1.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2008 growing season, one trial in soybean was conducted in a representative growing area in Northern France, to determine the residue level of bentazone, its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
BAS 762 01 H (480 g/L bentazone, SL) was applied once at a rate equivalent to 0.924 kg a.s./ha of bentazone and the spray volume used was 205 L/ha. The application was done at growth stage BBCH 13. Specimens of soybean samples (RAC seeds) were collected at harvest (BBCH 89).

Table 6.3/83 Target application rates and timings for soybeans

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2008	1	1	F	BAS 762 01 H (SL)	BAS 351 H	0.900	100-400	BBCH 12-25

2. Description of analytical procedures

The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg.
Samples were extracted with aqueous methanol, followed by iso-octane extraction and liquid/liquid partition of an aliquot. Hydrolysis was performed using enzymatic cleavage, and a $Ca(OH)_2$-precipitation step was included to remove acidic matrix constituents. Reversed phase C_{18}-SPE clean-up was performed before the final determination by liquid chromatography and tandem mass spectrometric detection (LC-MS/MS).

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3/84, detailed residue levels are shown in Table 6.3/85.
No residues of bentazone, 6-OH-bentazone and 8-OH-bentazone above the LOQ of 0.03 mg/kg could be detected after 90 DALA (BBCH 89).

Table 6.3/84 Summary of residues in soybeans

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	2008	**90**	**89**	**seeds**	**<0.01**	**<0.01**	**<0.01**	**<0.03**

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Total bentazone residues in seed specimens at BBCH 89 were below the LOQ of 0.03 mg/kg.

Table 6.3/85 Residues of bentazone in soybean after one application of BAS 762 01 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[5] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Benta-zone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	B89774	Soybean	France	BAS 762 01 H 1 x 0.924	13	90	seeds	<0.01	<0.01	<0.01	<u>≤0.03</u>	**Method: BASF Method No. 438/2**				
Doc ID:	2008/1034456											LOQ = 0.01 mg/kg				
Trial No.	A/NF/H/08/48											Bentazone				
GLP:	yes											seeds	0.01-0.1	2	96	N/A
Year	2008											6-OH-bentazone				
												seeds	0.01-0.1	2	85	N/A
												8-OH-bentazone				
												seeds	0.01-0.1	2	84	N/A

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
6) at application
_ underlined values were used for MRL calculation
N/A not applicable

Report:	II A 6.3.7/6 Kreke N. 2008(i) BAS 762 01 H with adjuvant BAS 9047 0S (DASH HC) - Determination of residues at harvest of Imazamox and Bentazone in soy bean (RAC seed) following one treatment with this tankmix from four open field trials, Italy and Southern France, 2007 BASF DocID 2007/1028359
Guidelines:	EEC 96/68; EEC 7029/VI/95 rev. 5
GLP:	Yes (laboratory certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	II A 6.3.7/7 Kreke N. 2010(s) First amendment: BAS 762 01 H with adjuvant BAS 9047 0S (DASH HC) - Residues at harvest of Imazamox and Bentazone in soy bean (RAC seed) following one treatment with this tankmix from four open field trials, Italy and Southern France, 2007 BASF DocID 2010/1155807
Guidelines:	EEC 96/68; EEC 7029/VI/95 rev. 5
GLP:	Yes (laboratory certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Executive Summary

During the 2007 growing season, four trials in soybean were conducted in representative growing areas in Italy and Southern France, to determine the residue level of bentazone, its metabolites 6-OH-bentazone and 8-OH-bentazone and imazamox with its metabolite CL 263384 in or on raw agricultural commodities (RAC). In the following only residue data from bentazone as well as of its metabolites 6-OH-bentazone and 8-OH-bentazone are presented.
BAS 762 01 H (480 g/L bentazone, SL) was applied once at a rate equivalent between 0.900 to 0.987 kg a.s./ha of bentazone together with an adjuvant BAS 9047 OS (DASH HC) and the spray volume used was 201-417 L/ha. The application was done at growth stage (GS) BBCH 13-24. Specimens of soybean samples (RAC seeds) were collected at harvest and analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. Total bentazone residues in seed specimens at BBCH 89 were below the LOQ of 0.03 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 762 01 H (SL), BAS 9047 0S (DASH HC); adjuvant (0.5% by volume of spray solution
Lot/Batch #:	402009, 480 g/L bentazone (nominal)
Purity:	Not reported
CAS#:	25057-89-0
Development code:	Not reported
Spiking levels:	0.01 mg/kg

2. Test Commodity:

Crop:	Soybean
Type:	Oilseeds
Variety:	Quito, Deka Big, M10 (Pioneer), B92 (Pioneer)
Botanical name:	*Glycine max.*
Crop parts(s) or processed commodity:	Seeds
Sample size:	min. 1.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2007 growing season, four trials in soybean were conducted in representative growing areas in Italy and Southern France, to determine the residue level of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
BAS 762 01 H (480 g/L bentazone, SL) was applied once at a rate equivalent between 0.900 to 0.987 kg a.s./ha of bentazone together with an adjuvant BAS 9047 OS (DASH HC) and the spray volume used was 201-417 L/ha. The application was done at growth stage (GS) BBCH 13-24. Specimens of soybean samples (RAC seeds) were collected at harvest (BBCH 89).

Table 6.3/86 Target application rates and timings for soybeans

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2007	4	1	F	BAS 762 01 H (SL)	BAS 351 H	0.900	100-400	BBCH 12-25

2. Description of analytical procedures

The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg.
Samples were extracted with aqueous methanol, followed by iso-octane extraction and liquid/liquid partition of an aliquot. Hydrolysis was performed using enzymatic cleavage, and a $Ca(OH)_2$-precipitation step was included to remove acidic matrix constituents. Reversed phase C_{18}-SPE clean-up was performed before the final determination by liquid chromatography and tandem mass spectrometric detection (LC-MS/MS).

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3/87, detailed residue levels are shown in Table 6.3/88.
Residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were between 85-104 DALA (BBCH 89) at <0.03 mg/kg.

Table 6.3/87 Summary of residues in soybeans

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU South	2007	**85-104**	**89**	**seeds**	**<0.01**	**<0.01**	**<0.01**	**<0.03**

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Total bentazone residues in seed specimens at BBCH 89 were below the LOQ of 0.03 mg/kg.

Table 6.3/88 Residues of bentazone in soybean after one application of BAS 762 01 H with adjuvant BAS 9047 0S (DASH HC) in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	B44111	Soybean	France	BAS 762 01 H: 1 x 0.987	13	90	seeds	<0.01	<0.01	<0.01	<u>≤0.03</u>	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2007/1028359											Bentazone				
	2010/1155807											seeds	0.01	1	119	N/A
Trial No.	A/SF/H/07/165											6-OH-bentazone				
GLP:	yes											seeds	0.01	1	110	N/A
Year	2007											8-OH-bentazone				
Study code:	B44111	Soybean	France	BAS 762 01 H: 1 x 0.906	13	104	seeds	<0.01	<0.01	<0.01	<u>≤0.03</u>	seeds	0.01	1	92	N/A
Doc ID:	2007/1028359															
	2010/1155807															
Trial No.	A/SF/H/07/166															
GLP:	yes															
Year	2007															
Study code:	B44111	Soybean	Italy	BAS 762 01 H: 1 x 0.900	23-24	88	seeds	<0.01	<0.01	<0.01	<u>≤0.03</u>					
Doc ID:	2007/1028359															
	2010/1155807															
Trial No.	A/IT/H/07/167															
GLP:	yes															
Year	2007															
Study code:	B44111	Soybean	Italy	BAS 762 01 H: 1 x 0.937	24	85	seeds	<0.01	<0.01	<0.01	<u>≤0.03</u>					
Doc ID:	2007/1028359															
	2010/1155807															
Trial No.	A/IT/H/07/168															
GLP:	yes															
Year	2007															

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone

3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
6) at application
_ underlined values were used for MRL calculation
N/A not applicable

Report:	II A 6.3.7/8 Kreke N. 2008(j) BAS 762 01 H - Determination of residues at harvest of Imazamox and Bentazone in soy bean (RAC seed) following one treatment with BAS 762 01 H (22.4/480 g/L Imazamox/Bentazone) from four open field trials in Italy and Southern France, 2007 BASF DocID 2007/1023134
Guidelines:	EEC 96/68; EEC 7029/VI/95 rev. 5
GLP:	Yes (laboratory certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	II A 6.3.7/9 Kreke N. 2010(t) First amendment: BAS 762 01 H - Determination of residues at harvest of Imazamox and Bentazone in soy bean (RAC seed) following one treatment with BAS 762 01 H (22.4/480 g/L) from four open field trials in Italy and Southern France in 2007 BASF DocID 2010/1155806
Guidelines:	EEC 96/68; EEC 7029/VI/95 rev. 5
GLP:	Yes (laboratory certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Executive Summary

During the 2007 growing season, four trials in soybean were conducted in a representative growing area in Italy and Southern France, to determine the residue level of bentazone, its metabolites 6-OH-bentazone and 8-OH-bentazone and imazamox with its metabolite CL 263384 in or on raw agricultural commodities (RAC). In the following only residue data from bentazone as well as of its metabolites 6-OH-bentazone and 8-OH-bentazone are presented here.
BAS 762 01 H (480 g/L bentazone, SL) was applied once at a rate equivalent between 0.866 to 0.960 kg a.s./ha of bentazone and the spray volume used was 193-410 L/ha. The application was done at growth stage (GS) BBCH 13-24. Specimens of soybean samples (RAC seeds) were collected at harvest and analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. Total bentazone residues in seed specimens at BBCH 89 were below the LOQ.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 762 01 H (SL)
Lot/Batch #:	402009, 480 g/L bentazone (nominal)
Purity:	Not reported
CAS#:	25057-89-0
Development code:	Not reported
Spiking levels:	0.01-0.1 mg/kg

2. Test Commodity:

Crop:	Soybean
Type:	Oilseeds
Variety:	Quito, Deka Big, M10 (Pioneer), B92 (Pioneer)
Botanical name:	*Glycine max.*
Crop parts(s) or processed commodity:	Seeds
Sample size:	min. 1.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2007 growing season, four trials in soybean were conducted in representative growing area in Italy and Southern France to determine the residue level of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
BAS 762 01 H (480 g/L bentazone, SL) was applied once at a rate equivalent between 0.866 to 0.960 kg a.s./ha of bentazone and the spray volume used was 193-410 L/ha. The application was done at growth stage BBCH 13-24. Specimens of soybean samples (RAC seeds) were collected at harvest (BBCH 89).

Table 6.3/89 Target application rates and timings for soybeans

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2007	4	1	F	BAS 762 01 H (SL)	BAS 351 H	0.900	100-400	BBCH 12-25

2. Description of analytical procedures

The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg.
Samples were extracted with aqueous methanol, followed by iso-octane extraction and liquid/liquid partition of an aliquot. Hydrolysis was performed using enzymatic cleavage, and a $Ca(OH)_2$-precipitation step was included to remove acidic matrix constituents. Reversed phase C_{18}-SPE clean-up was performed before the final determination by liquid chromatography and tandem mass spectrometric detection (LC-MS/MS).

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3/90, detailed residue levels are shown in Table 6.3/91.
Residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were between 85-104 DALA (BBCH 89) at <0.03 mg/kg.

Table 6.3/90 Summary of residues in soybeans

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU South	2007	**85-104**	**89**	**seeds**	**<0.01**	**<0.01**	**<0.01**	**<0.03**

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Total bentazone residues in seed specimens at BBCH 89 were below LOQ.

Table 6.3/91 Residues of bentazone in soybean after one application of BAS 762 01 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	B42941	Soybean	France	BAS 762 01 H: 1 x 0.960	13	90	seeds	<0.01	<0.01	<0.01	<u>≤0.03</u>	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2007/1023134											Bentazone				
	2010/1155806											seeds	0.01, 0.1	2	105	N/A
Trial No.	A/SF/H/07/142											6-OH-bentazone				
GLP:	yes											seeds	0.01, 0.1	2	65	N/A
Year	2007											8-OH-bentazone				
Study code:	B42941	Soybean	France	BAS 762 01 H: 1 x 0.866	13	104	seeds	<0.01	<0.01	<0.01	<u>≤0.03</u>	seeds	0.01	1	100	N/A
Doc ID:	2007/1023134															
	2010/1155806															
Trial No.	A/SF/H/07/143															
GLP:	yes															
Year	2007															
Study code:	B42941	Soybean	Italy	BAS 762 01 H: 1 x 0.923	23-24	88	seeds	<0.01	<0.01	<0.01	<u>≤0.03</u>					
Doc ID:	2007/1023134															
	2010/1155806															
Trial No.	A/IT/H/07/144															
GLP:	yes															
Year	2007															

Table 6.3/91 Residues of bentazone in soybean after one application of BAS 762 01 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	B42941	Soybean	Italy	BAS 762 01 H: 1 x 0.900	24	85	seeds	<0.01	<0.01	<0.01	<u>≤0.03</u>					
Doc ID:	2007/1023134 2010/1155806															
Trial No.	A/IT/H/07/145															
GLP:	yes															
Year	2007															

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
6) at application
_ underlined values were used for MRL calculation
N/A not applicable

Linseed

Table 6.3/92 GAP for the use of BAS 351 H in/on linseed

Crop	Maximum Applied Dose	Water Volume	PHI	Application Method	Application Timing
Linseed	1 x 0.96 kg BAS 351 H/ha	200-400 L/ha	F	spray application	-

PHI = pre-harvest interval

Table 6.3/93 GAP information of residue trials conducted in linseed in 1999

Region	Country	Formulation	Application[0]				DALA[1]
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
EU South	France (4 trials)	BAS 351 32 H, SL	spray application	1.483-1.601	0.512	1	28-34 97-103 97-103

0) actual application rates varied by 10% at most
1) days after last application

Report: II A 6.3.7/10
Blaschke U.G. 2000(i)
Determination of the magnitude of the residue of BAS 351 32 H in/on flax raw agricultural commodity specimens from supervised field trials in Southern Europe in 1999
BASF DocID 2000/1018491

Guidelines: FAO Guidelines Rome 1990; EEC 96/68; EEC 87/18; EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8)

GLP: Yes
(laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

A total number of four field trials were conducted in representative linseed growing areas in Southern France (EU-S) in 1999. The herbicidal product BAS 351 32 H (485 g/L BAS 351 H, SL) was applied one time at an application rate of 3.2 L/ha corresponding to an application rate of 1.55 kg a.s./ha. The first samples (shoots) were collected 28-34 days after the last application and subsequent samples (straw and seeds) were taken after 97-103 days post application. Specimens were analysed by means of BASF Method No. 438/1 (which is a miniaturised version of BASF Method No. 438/0).
The limit of quantification was 0.02 mg/kg for all analysed substances. The study showed that no quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone in linseed seeds were detectable. This is resulting in a total bentazone residue level (expressed as bentazone equivalents) below 0.06 mg/kg. Therefore, the residue values were used for MRL determination (see chapter 6.7) even though an exaggerated application rate compared to the critical GAP was used.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 32 H (SL)
Lot/Batch #:	98-1, 480 g/L bentazone (nominal)
Purity:	Not reported
CAS#:	25057-89-0
Development code:	Not reported
Spiking levels:	0.02-2.0 mg/kg

2. Test Commodity:

Crop:	Linseed
Type:	Oilseeds
Variety:	Mikael, Ceres
Botanical name:	*Linum usitatissimum*
Crop parts(s) or processed commodity:	Shoots, seeds, straw
Sample size:	Not reported

B. STUDY DESIGN

1. Test procedure A total number of 4 supervised field trials were conducted in different representative linseed growing areas in Southern France (EU-S) in 1999. The herbicidal product BAS 351 32 H (480 g/L BAS 351 H, SL) was applied once of about 3.2 L/ha corresponding to an application rate of 1.55 kg a.s./ha. The first samples (shoots) were collected 28-34 days after the last application and subsequent samples (straw and seeds) were taken after 97-103 days post application. Collected samples were stored frozen at approximately -20°C until further analysis.

Table 6.3/94 Target application rates and timings for linseed

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
1999	4 (S)	1	F	BAS 351 32 H (SL)	BAS 351 H	1.552	300	BBCH 13-15

2. Description of analytical procedures

Residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were determined following BASF Method No. 438/1 (which is a miniaturised version of BASF Method No. 438/0). Bentazone and its metabolites are extracted from plant matrices with aqueous methanol. After purification by an iso-octane liquid/liquid partition, the two relevant metabolites which are present as glucosides are hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic plant constituents, a reversed phase C_{18}-column clean-up is performed. The analytes are then methylated with diazomethane and their derivatives are purified using a silica gel-column. The final determination of the residues of bentazone and its hydroxy metabolites is performed by GC-MS. The limit of quantification was 0.02 mg/kg for all analysed substances.

II. RESULTS AND DISCUSSION

The residues of bentazone, 6-OH-bentazone and 8-OH-bentazone in soybeans treated one time with the single formulation BAS 351 32 F are shown in Table 6.3/95.
The measured residues of bentazone and 8-OH-bentazone in linseeds plant treated one time with BAS 351 32 H were below the limit of detection of <0.02 mg/kg in each analysed sample (i.e. shoots, straw and seeds). The measured residual concentrations of 6-OH-bentazone ranged from 0.104 to 0.432 mg/kg in shoots and from 0.106 to 0.258 mg/kg in straw, whereas no residues where detectable in the linseed seeds. Total residues of bentazone (calculated as bentazone equivalents) were between 0.14 to 0.45 mg/kg in shoots and between 0.14 to 0.28 mg/kg in straw. In linseed seeds, the total bentazone residues were calculated to be <0.06 mg/kg.

Table 6.3/95 Summary of residues in linseed

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU South	1999	28-34	31-32	shoots	<0.02	0.104-0.434	<0.02	0.14-0.45
		97-103	**89**	**seeds**	**<0.02**	**<0.02**	**<0.02**	**<0.06**
		97-103	89	straw	<0.02	0.106-0.258	<0.02	0.14-0.28

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

The study showed that no quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone in linseed seeds were detectable, resulting in a total bentazone residue (expressed as bentazone equivalents) below 0.06 mg/kg.

Table 6.3/96 Residues of bentazone in linseed after one application of BAS 351 32 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	BSF 607/002849	Linseed	France	BAS 351 32 H: 1 x 1.483	15	34	shoots	<0.02	0.104	<0.02	0.14	**Method:** No. 438/1; LOQ = 0.02 mg/kg				
Doc ID:	2000/1018491					97	seeds	<0.02	<0.02	<0.02	<0.06	Bentazone				
Trial No.	BSF/607-1					97	straw	<0.02	0.258	<0.02	0.28	shoots	0.02-2.0	3	81	20
GLP:	yes											seeds	0.02.0.2	2	99.1	N/A
Year	1999											straw	0.02-0.2	2	108.4	N/A
Study code:	BSF 607/002849	Linseed	France	BAS 351 32 H: 1 x 1.527	14	28	shoots	<0.02	0.179	<0.02	0.21	6-OH-bentazone				
Doc ID:	2000/1018491					103	seeds	<0.02	<0.02	<0.02	<0.06	shoots	0.2-2.0	2	66.3	N/A
Trial No.	BSF/607-2					103	straw	<0.02	0.119	<0.02	0.15	seeds	0.02.0.2	2	85.1	N/A
GLP:	yes											straw	0.02-0.2	2	95.2	N/A
Year	1999											8-OH-bentazone				
Study code:	BSF 607/002849	Linseed	France	BAS 351 32 H: 1 x 1.595	13	29	shoots	<0.02	0.434	<0.02	0.45	shoots	0.02-2.0	2	86.7	N/A
Doc ID:	2000/1018491					101	seeds	<0.02	<0.02	<0.02	<0.06	seeds	0.02.0.2	2	80.3	N/A
Trial No.	BSF/607-3					101	straw	<0.02	0.135	<0.02	0.17	straw	0.02-0.2	2	91.4	N/A
GLP:	yes															
Year	1999															
Study code:	BSF 607/002849	Linseed	France	BAS 351 32 H: 1 x 1.601	13	29	shoots	<0.02	0.432	<0.02	0.44					
Doc ID:	2000/1018491					101	seeds	<0.02	<0.02	<0.02	<0.06					
Trial No.	BSF/607-4					101	straw	<0.02	0.106	<0.02	0.14					
GLP:	yes															
Year	1999															

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.02 + 0.02 + 0.02 = 0.06; 0.02 + <0.02 + <0.02 = 0.06; <0.02 + <0.02 + <0.02 = <0.06
6) at application
_ underlined values were used for MRL calculation
N/A not applicable

6.3.8 Cereals

Cereals

Table 6.3/97 GAP for the use of BAS 351 H in/on cereals

Crop	Maximum Applied Dose	Water Volume	PHI	Application Method	Application Timing
Maize	1 x 0.96 kg BAS 351 H/ha	200-400 L/ha	F	spray application	BBCH 11-18
Barley	1 x 0.96 kg BAS 351 H/ha	200-400 L/ha	F	spray application	BBCH 12-31
Wheat	1 x 0.96 kg BAS 351 H/ha	200-400 L/ha	F	spray application	BBCH 12-31

PHI = pre-harvest interval

Table 6.3/98 GAP information of residue trials conducted in cereals between 1998 and 2010

Crop	Region	Country	Formulation	Application[0]				DALA[1]
				Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
Maize	EU North	Germany (1 trial) The Netherlands (1 trial)	BAS 351 40 H, SL	spray application	0.96	0.48	1	0 8-18 60 99-106
		United Kingdom (1 trial)	BAS 351 40 H, SL	spray application	0.96	0.48	1	0 18 71 60 89
		France (1 trial) Germany (1 trial)	BAS 351 40 H, SL	spray application	1.2	0.6	1	0 19 27-28 62
		France (1 trial) United Kingdom (2 trials)	BAS 351 40 H, SL	spray application	1.2	0.6	1	0 24 28-29 36-48 55-61 69-89
		The Netherlands (1 trial) Germany (1 trial)	BAS 600 00 H, SC	spray application	0.8	0.26	1	0 28-36 103-117 138-142
		France (2 trials)	BAS 351 40 H, SL	spray application	1.54	0.616	1	0 30-45 76-115 107-140

Table 6.3/98 GAP information of residue trials conducted in cereals between 1998 and 2010

Crop	Region	Country	Formulation	Application[0]				DALA[1]
				Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
Maize	EU South	Italy (1 trial) Spain (1 trial)	BAS 351 40 H, SL	spray application	0.96	0.48	1	0 14-34 56-60 60-69 84-124
		France (1 trial)	BAS 351 40 H, SL	spray application	0.96	0.48	1	0 27 61 119
		France (1 trial) Spain (1 trial)	BAS 351 40 H, SL	spray application	1.2	0.6	1	0 28 63-64 76
		Italy (1 trial) Spain (2 trials)	BAS 351 40 H, SL	spray application	1.2	0.6	1	0 28 35-42 42-50 65-96
		France (2 trials)	BAS 351 40 H, SL	spray application	1.54	0.616	1	0 46-48 108-111 141-144
		Spain (3 trials) Italy (3 trials)	BAS 351 32 H, SL	spray application	1.55	0.517	1	0 23-44 68-97 100-127
Barley	EU South	Spain (2 trials) Italy (3 trials)	BAS 351 32 H, SL	spray application	1.55	0.517	1	0 17-26 57-65
Wheat	EU North	Germany (2 trials) United Kingdom (2 trials) France (2 trials) The Netherlands (1 trial) Denmark (1 trial)	BAS 351 32 H, SL	spray application	0.96	0.48	1	0 22-36 59-67 87-103
	EU South	Spain (2 trials) Italy (2 trials)	BAS 351 32 H, SL	spray application	1.55	0.517	1	0 16-28 70-78

0) actual application rates varied by 10% at most
1) days after last application

Maize

Report:	II A 6.3.8/1 Oxspring S. 2011(a) Study on the behaviour of Bentazone in maize after treatment with BAS 351 32 H in Northern and Southern Europe during 2010 BASF DocID 2011/1059496
Guidelines:	None
GLP:	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

During the 2010 growing season, six field trials were conducted in representative maize growing areas in the Northern and Southern Europe to determine the residue level of bentazone in or on raw agricultural commodities (RAC). Plot 2 was treated with BAS 351 32 H, an SL formulation of bentazone (480 g a.s./L) and was foliar applied once at a rate equivalent to 0.96 kg a.s./ha to maize at BBCH 35. Untreated and treated maize whole plant specimens were sampled immediately after the application and at BBCH 51 at all trials. Cobs with husks and rest of plant specimens were sampled at about 60 DALA and additionally at BBCH 85 at some trials. At other trials the BBCH 85 sampling was not realised. Specimens of maize grain and rest of plant were sampled at BBCH 89 at all trials.
All maize specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone according to BASF Analytical Method 438/2 (L0044/02) which determines the analytes by means of LC-MS/MS with a limit of quantitation (LOQ) of 0.01 mg/kg. At harvest (BBCH 89), 85-124 days after the application, total bentazone residues in maize grain were below the LOQ of 0.03 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 40 H (SL)
Lot/Batch #:	70896356PO, 480 g a.s./L nominal
Purity:	
CAS#:	25057-89-0
Development code:	
Spiking levels:	0.01-40 mg/kg

2. Test Commodity:

Crop:	Maize
Type:	Cereals
Variety:	Franz, N K Cheer, Aabsint, KWS 6565, Tyrex, Mitic
Botanical name:	*Zea mays*
Crop parts(s) or processed commodity:	Whole plant without roots, cobs with husks, rest of plant, grain
Sample size:	≥0.5-2.0 kg / 12 units

B. STUDY DESIGN

1. Test procedure

During the 2010 growing season, six field trials were conducted in representative maize growing areas in the UK, Germany, France, The Netherlands, Italy and Spain to determine the residue level of bentazone in or on raw agricultural commodities (RAC).

Plot 2 was treated with BAS 351 32 H, an SL formulation of bentazone (480 g a.s./L) and was foliar applied once at a rate of 2 L of formulated product/ha (equates to 0.96 kg a.s./ha) to maize at BBCH 35. The nominal spray volume used was 200 L/ha.

Untreated and treated maize whole plant specimens were sampled immediately after the application and at BBCH 51 at all trials. Untreated plots were sampled before treated plots. Untreated and treated maize (cobs with husks, rest of plant) specimens were sampled at 60±1 DALA and additionally at BBCH 85 at trials L100120, L100122 and L100124. At trials L100119 and L100121 the BBCH 85 sampling was not realised, and at trial L100123 the 60±1 DALA and BBCH 85 sampling timings coincided. Specimens of maize grain and rest of plant were sampled at BBCH 89 at all trials. Samples were stored frozen at or below -18°C until analysis. The maximum storage interval was 369 days.

Table 6.3/99: Target application rates and timings for maize

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2010	3 (N-EU) 3 (S-EU)	1	F	BAS 351 40 H (SL)	BAS 351 H	0.960	200	BBCH 35

2. Description of analytical procedures

All maize specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone according to BASF Analytical Method 438/2 (L0044/02) which determines the analytes by means of LC-MS/MS with a limit of quantitation (LOQ) of 0.01 mg/kg.

Bentazone and its metabolites are extracted from plant matrices with aqueous methanol. After purification by an iso-octane liquid-liquid partition, hydrolysis using enzymatic cleavage is performed. After a $Ca(OH)_2$-precipitation step to remove acidic plant constituents, a reversed phase C_{18}-SPE clean-up is performed. The final determination of the residues of bentazone and its hydroxy metabolites is performed by LC-MS/MS.

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3/100, detailed residue levels are shown in Table 6.3/101 and Table 6.3/102.
The bentazone residues in maize whole plant without roots specimens taken directly after the application ranged between 6.2 and 31 mg/kg. At BBCH 51 the residues had declined to a range of <0.01 to 0.03 mg/kg. At 56-61 DALA and at BBCH 85, no residues of bentazone were determined in the specimens of cob and rest of plant (<0.01 mg/kg). At harvest (BBCH 89) no residues above the LOQ were found in the specimens of grain and rest of plant.
The 6-OH-bentazone residues (expressed as bentazone) in maize whole plant without roots specimens taken directly after the application ranged between 0.45 and 8.9 mg/kg. At BBCH 51 the residues ranged between 0.19 and 3.3 mg/kg. At 56-61 DALA and BBCH 85, residues between 0.05 and 1.5 mg/kg were detected in the rest of plant specimens, whereas no residues were detected in the cob specimens. At harvest (BBCH 89) residues of between <0.01 and 1.1 mg/kg were detected in the rest of plant specimens, with no residues (<0.01 mg/kg) detected in the grain specimens.
The 8-OH-bentazone residues (expressed as bentazone) in maize whole plant without roots specimens taken directly after the application ranged between 0.11 and 0.82 mg/kg. At BBCH 51 the residues ranged between <0.01 and 0.04 mg/kg. At 56-61 DALA and BBCH 85, residues between <0.01 and 0.03 mg/kg were detected in the rest of plant specimens whereas no residues were detected in the cob specimens. At harvest (BBCH 89), residues between <0.01 and 0.01 mg/kg were detected in the rest of plant specimens, with no residues above the LOQ detected in the grain specimens.
The total bentazone residues in maize whole plant without roots specimens taken directly after the application ranged between 12 and 32 mg/kg. At BBCH 51 the residues in whole plant (w/o roots) specimens ranged between 0.21 and 3.3 mg/kg. At 56-61 DALA and at BBCH 85, residues between 0.07 and 1.5 mg/kg were determined in the specimens of rest of plant, whereas no residues were determined in the cob specimens. At harvest (BBCH 89), residues between <0.03 and 1.1 mg/kg of total bentazone were determined in the rest of plant specimens, whereas no residues (<0.03 mg/kg) were detected in the grain specimens.
No residues of bentazone or its metabolites 6-OH-bentanzone and 8-OH-bentazone above the limit of quantitation (0.01 mg/kg) were detected in the control specimens of this study, with the exception of sample number L1001190001 (0.01 mg/kg bentazone).

Table 6.3/100: Summary of residues in maize

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	2010	0	35	whole plant[5]	12-24	0.50-8.9	0.11-0.18	17-25
		8-18	51	whole plant[5]	<0.01-0.03	1.5-3.3	0.01-0.04	1.5-3.3
		60	75-83	cobs with husks	<0.01	<0.01	<0.01	<0.03
		71	85	cobs with husks	<0.01	<0.01	<0.01	<0.03
		60	75-83	rest of plant	<0.01	0.72-1.5	0.01-0.03	0.74-1.5
		71	85	rest of plant	<0.01	1.2	0.02	1.3
		89-106	89	rest of plant	<0.01	0.10-1.1	<0.01-0.01	0.12-1.1
		89-106	**89**	**grain**	**<0.01**	**<0.01**	**<0.01**	**<0.03**
EU South		0	35	whole plant[5]	6.2-31	0.45-6.0	0.20-0.82	12-32
		14-34	51-53	whole plant[5]	<0.01	0.19-0.22	<0.01	0.21-0.24
		56-61	83-85	cobs with husks	<0.01	<0.01	<0.01	<0.03
		69	85	cobs with husks	<0.01	<0.01	<0.01	<0.03
		56-61	83-85	rest of plant	<0.01	0.10-0.15	<0.01	0.12-0.17
		69	85	rest of plant	<0.01	0.05	<0.01	0.07
		85-124	89	rest of plant	<0.01	<0.01	<0.01	<0.01
		85-124	**89**	**grain**	**<0.01**	**<0.01**	**<0.01**	**<0.03**

1) days after last application
2) expressed as parent equivalent
3) expressed as parent equivalent
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) without roots

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

At harvest (BBCH 89), 85-124 days after the application, total bentazone residues in maize grain were below the LOQ of 0.03 mg/kg.

Table 6.3/101: Residues of bentazone in maize after one application of BAS 351 40 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	385354	Maize	Germany	BAS 351 40 H: 1 x 0.96	35	0	plant[5]	12	8.9	0.11	21	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2011/1059496					8	plant[5]	0.03	3.3	0.04	3.3	Bentazone				
Trial No.	L100119					60	cobs[7]	<0.01	<0.01	<0.01	<0.03	plant[5]	0.01-40	5	101	6.7
GLP:	yes					60	rest plant[5]	<0.01	0.74	0.03	*0.77*	cobs[7)]	0.01-0.10	4	106	1.2
Year	2010					99	grain	<0.01	<0.01	<0.01	<0.03	grain	0.01-0.10	4	109	0.9
						99	rest plant[5]	<0.01	0.10	<0.01	0.12	rest plant	0.01-0.10	4	106	1.8
Study code:	385354	Maize	United Kingdom	BAS 351 40 H: 1 x 0.96	35	0	plant[5]	16	0.50	0.14	17	6-OH-bentazone				
Doc ID:	2011/1059496					18	plant[5]	0.02	1.6	0.01	1.6	plant[5]	0.01-9.6	5	101	5.2
Trial No.	L100120					71	cobs[7]	<0.01	<0.01	<0.01	<0.03	cobs[7)]	0.01-0.10	4	82	14
GLP:	yes					71	rest plant[5]	<0.01	1.2	0.02	*1.3*	grain	0.01-0.10	4	96	1.8
Year	2010					60	cobs[7]	<0.01	<0.01	<0.01	<0.03	rest plant	0.01-0.10	4	97	10
						60	rest plant[5]	<0.01	1.5	0.02	1.5	8-OH-bentazone				
						89	grain	<0.01	<0.01	<0.01	<0.03	plant[5]	0.01-0.96	5	102	5.9
						89	rest plant[5]	<0.01	1.1	0.01	1.1	cobs[7)]	0.01-0.10	4	85	13
Study code:	385354	Maize	The Netherlands	BAS 351 40 H: 1 x 0.96	35	0	plant[5]	24	0.73	0.18	25	grain	0.01-0.10	4	99	3.8
Doc ID:	2011/1059496					18	plant[5]	<0.01	1.5	0.02	1.5	rest plant	0.01-0.10	4	104	1.6
Trial No.	L100121					60	cobs[7]	<0.01	<0.01	<0.01	<0.03					
GLP:	yes					60	rest plant[5]	<0.01	0.72	0.01	*0.74*					
Year	2010					106	grain	<0.01	<0.01	<0.01	<0.03					
						106	rest plant[5]	<0.01	0.39	<0.01	0.41					

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as parent equivalent, the conversion factor for both metabolites is 0.938;
3) expressed as parent equivalent; the conversion factor for both metabolites is 0.938;
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) at application

7) with husks
_ underlined values were used for MRL calculation
values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3/102: Residues of bentazone in maize after one application of BAS 351 40 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	385354	Maize	Italy	BAS 351 40 H: 1 x 0.96	35	0	plant[5]	31	0.45	0.82	32	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2011/1059496					34	plant[5]	<0.01	0.19	<0.01	0.21	Bentazone				
Trial No.	L100122					69	cobs[7]	<0.01	<0.01	<0.01	<0.03	plant[5]	0.01-40	5	101	6.7
GLP:	yes					69	rest plant[5]	<0.01	0.05	<0.01	*0.07*	cobs[7)]	0.01-0.10	4	106	1.2
Year	2010					60	cobs[7]	<0.01	<0.01	<0.01	<0.03	grain	0.01-0.10	4	109	0.9
						60	rest plant[5]	<0.01	0.11	<0.01	0.13	rest plant	0.01-0.10	4	106	1.8
						84	grain	<0.01	<0.01	<0.01	<u>≤0.03</u>	6-OH-bentazone				
						84	rest plant[5]	<0.01	<0.01	<0.01	<0.03	plant[5]	0.01-9.6	5	101	5.2
Study code:	385354	Maize	France	BAS 351 40 H: 1 x 0.96	35	0	plant[5]	6.2	6.0	0.20	12	cobs[7)]	0.01-0.10	4	82	14
Doc ID:	2011/1059496					27	plant[5]	<0.01	0.22	<0.01	0.24	grain	0.01-0.10	4	96	1.8
Trial No.	L100123					61	cobs[7]	<0.01	<0.01	<0.01	<0.03	rest plant	0.01-0.10	4	97	10
GLP:	yes					61	rest plant[5]	<0.01	0.12	<0.01	*0.14*	8-OH-bentazone				
Year	2010					119	grain	<0.01	<0.01	<0.01	<u>≤0.03</u>	plant[5]	0.01-0.96	5	102	5.9
						119	rest plant[5]	<0.01	<0.01	<0.01	<0.03	cobs[7)]	0.01-0.10	4	85	13
Study code:	385354	Maize	Spain	BAS 351 40 H: 1 x 0.96	35	0	plant[5]	12	0.8	0.42	14	grain	0.01-0.10	4	99	3.8
Doc ID:	2011/1059496					14	plant[5]	<0.01	0.20	<0.01	0.22	rest plant	0.01-0.10	4	104	1.6
Trial No.	L100124					56	cobs[7]	<0.01	<0.01	<0.01	<0.03					
GLP:	yes					56	rest plant[5]	<0.01	0.15	<0.01	0.17					
Year	2010					60	cobs[7]	<0.01	<0.01	<0.01	<0.03					
						60	rest plant[5]	<0.01	0.10	<0.01	*0.12*					
						124	grain	<0.01	<0.01	<0.01	<u>≤0.03</u>					
						124	rest plant[5]	<0.01	<0.01	<0.01	<0.03					

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as parent equivalent, the conversion factor for both metabolites is 0.938;
3) expressed as parent equivalent; the conversion factor for both metabolites is 0.938;
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
   for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) at application
7) with husks
_ underlined values were used for MRL calculation
values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Report:	II A 6.3.8/2 Klaas P., Ziske J. 2009(b) Study on the residue behaviour of Bentazone in maize after treatment with BAS 351 40 H under field conditions in Germany, Northern France, Southern France and Spain, 2008 BASF DocID 2009/1024805
Guidelines:	EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8); EEC 1607/VI/97 rev. 2 10.06.1999; EEC 7029/VI/95 rev. 5; EEC 7525/VI/95 rev. 7
GLP:	Yes (laboratory certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Executive Summary

During the 2008 growing season, 4 trials in maize (field conditions) were conducted in different representative growing areas in Germany, Northern and Southern France and Spain to determine the residue level of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
BAS 351 40 H (480.0 g/L bentazone, SL) was applied once at a rate equivalent to 1.2 kg a.s./ha of bentazone. The application was done at BBCH 55.
Sampling was carried out four or five times, depending on crop maturity. Specimens of whole plant without roots were collected at the day of the application and at 19 and 27-28 DALA. Cobs with husks and rest of plant with husks (no roots) specimens were collected at 19 and 27-28 DALA. Grain and rest of plant (no roots) specimens were collected at 62-64 DALA or in case of immaturity an additional sampling was done at growth stage BBCH 89.
The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg. Total bentazone residues grain specimens in all samples at harvest were below 0.03 mg/kg.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 40 H (SL)
Lot/Batch #:	12664, 480.0 g/L bentazone, nominal
Purity:	
CAS#:	25057-89-0
Development code:	
Spiking levels:	0.01-1 mg/kg

2. Test Commodity:

Crop:	Maize
Type:	Cereals
Variety:	Aspeed, Delitop, Mitic, Tardio 130
Botanical name:	*Zea mays*
Crop parts(s) or processed commodity:	- Whole plants without roots, cobs with husks, rest of plant with husks (no roots), grain, rest of plant (no roots)
Sample size:	1.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2008 growing season, 4 trials in maize (field conditions) were conducted in different representative growing areas in Germany, Northern and Southern France and Spain to determine the residue level of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
BAS 351 40 H (480.0 g/L bentazone, SL) was applied once at a rate equivalent to 1.2 kg a.s./ha of bentazone. The spray volume used was 200 L/ha and the application was done at BBCH 55.
Sampling was carried out four or five times, depending on crop maturity. Specimens of whole plant without roots were collected at the day of the application and at 19 and 27-28 DALA. Cobs with husks and rest of plant with husks (no roots) specimens were collected at 19 and 27-28 DALA. Grain and rest of plant (no roots) specimens were collected at 62-64 DALA or in case of immaturity an additional sampling was done at growth stage BBCH 89.

Table 6.3/103 Target application rates and timings for maize

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2008	2 (N-EU) 2 (S-EU)	1	F	BAS 351 40 H (SL)	BAS 351 H	1.2	200	BBCH 55

2. Description of analytical procedures

The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg.
Samples were extracted with aqueous methanol, followed by iso-octane extraction and liquid/liquid partition of an aliquot. Hydrolysis was performed using enzymatic cleavage, and a $Ca(OH)_2$-precipitation step was included to remove acidic matrix constituents. Reversed phase C_{18}-SPE clean-up was performed before the final determination by liquid chromatography and tandem mass spectrometric detection (LC-MS/MS).

II. RESULTS AND DISCUSSION

The residue ranges for the different trials are shown in Table 6.3/104, detailed residue levels are shown in Table 6.3/105 and Table 6.3/106.
The bentazone residues in maize whole plant specimens taken directly after the application ranged between 0.03-17.68 mg/kg. At 62-64, 76 and 105 DALA, residues in grain were below the LOQ of 0.01 mg/kg.
The 6-OH-bentazone residues in whole plant specimens taken directly after the application ranged between 0.025-5.79 mg/kg. At 62-64, 76 and 105 DALA residues were below the LOQ (<0.01 mg/kg).
The 8-OH-bentazone residues in maize whole plant without roots specimens taken directly after the application ranged between <0.01-1.08 mg/kg. At 62-64, 76 and 105 DALA residues also were below the LOQ (<0.01 mg/kg).
The total bentazone residues in maize whole plant without roots specimens taken directly after the application ranged between 0.65-23.47 mg/kg. At harvest the residues declined to levels below the LOQ (<0.03 mg/kg). Rest of plant without root specimens declined from 2.18 mg/kg (19 DALA) to 0.29 mg/kg (105 DALA).
No residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone above the limit of quantitation (0.01 mg/kg) were found in the control specimens of this study except one untreated specimen of rest of plant (6-OH-bentazone; 0.76 mg/kg, 8-OH-bentazone: 0.02 mg/kg).

Table 6.3/104 Summary of residues in maize

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	2008	0	55	whole plant[5]	12.56-17.68	4.71-5.79	0.95-1.08	19.3-23.47
		19	73-75	whole plant[5]	<0.01	2.16	<0.01	2.18
		27-28	75-77	whole plant[5]	<0.01	0.66-0.88	<0.01	0.68-0.90
		19	73-75	cobs w/o husks	<0.01	<0.01	<0.01	<0.03
		27-28	75-77	cobs w/o husks	<0.01	<0.01	<0.01	<0.03
		27-28	75-77	rest w husks	<0.01	1.00-1.61	<0.01	1.02-1.63
		62-63	**85-89**	**grain**	**<0.01**	**<0.01**	**<0.01**	**<0.03**
		105	**89**	**grain**	**<0.01**	**<0.01**	**<0.01**	**<0.03**
		19	73-75	rest of plant[5]	<0.01	2.16	<0.01	2.18
		62-63	85-89	rest of plant[5]	<0.01-0.01	<0.01-0.47	<0.01	<0.03-0.49
		105	89	rest of plant[5]	0.03	0.25	<0.01	0.29
EU South		0	55	whole plant[5]	0.03-5.58	0.025-2.03	<0.01-0.29	0.65-7.90
		28	73-75	whole plant[5]	<0.01	0.67-1.78	<0.01	0.69-1.80
		28	73-75	cobs w/o husks	<0.01	0.02-0.08	<0.01	0.04-0.10
		28	73-75	rest w husks	0.01-0.02	2.36-2.63	<0.01	2.38-2.66
		63-64	**87-89**	**grain**	**<0.01**	**<0.01**	**<0.01**	**<0.03**
		76	**89**	**grain**	**<0.01**	**<0.01**	**<0.01**	**<0.03**
		63-64	87-89	rest of plant[5]	<0.01-0.02	0.37-1.26	<0.01-0.01	0.39-1.29
		76	89	rest of plant[5]	0.04	1.03	<0.01	1.08

1) days after last application
2) expressed as parent equivalent
3) expressed as parent equivalent
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) without roots

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Total bentazone residues grain specimens in all samples at harvest were below 0.03 mg/kg.

Table 6.3/105 Residues of bentazone in maize after one application of BAS 351 40 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	347929	Maize	France	BAS 351 40 H: 1 x 1.2	55	0	plant[5]	17.68	4.71	1.08	23.47	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2009/1024805					27	cobs w/o husks	<0.01	<0.01	<0.01	**<0.03**	Bentazone				
Trial No.	L080404					27	rest w. husks	<0.01	1.00	<0.01	1.02	whole plant[5]	0.01-1.0	6	87.0	6.7
GLP:	yes					27	plant[5]	<0.01	0.66	<0.01	0.68	cobs with husks	0.01-1.0	3	87.2	6.2
Year	2008					63	grain	<0.01	<0.01	<0.01	<0.03	grain	0.01-1.0	3	88.5	7.8
						63	rest plant[5]	0.01	0.47	<0.01	0.49	rest of plant with husks[5]	0.01-1.0	3	87.5	1.4

Table 6.3/105 Residues of bentazone in maize after one application of BAS 351 40 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	347929	Maize	Germany	BAS 351 40 H: 1 x 1.2	55	0	plant[5]	12.56	5.76	0.95	19.30	rest of plant[5]	0.01-1.0	3	93.4	2.4
Doc ID:	2009/1024805					19	cobs w/o husks	<0.01	<0.01	<0.01	<0.03	6-OH-bentazone				
Trial No.	L080405					19	rest w. husks	<0.01	2.16	<0.01	2.18	whole plant[5]	0.01-1.0	6	99.9	6.2
GLP:	yes					19	plant[5]	<0.01	2.16	<0.01	2.18	cobs with husks	0.01-1.0	3	104.1	7.8
Year	2008					28	cobs w/o husks	<0.01	<0.01	<0.01	**<0.03**	grain	0.01-1.0	3	109.8	7.9
						28	rest w. husks	<0.01	1.61	<0.01	1.63	rest of plant with husks[5]	0.01-1.0	3	98.1	4.4
						28	plant[5]	<0.01	0.88	<0.01	0.90	rest of plant[5]	0.01-1.0	3	101.7	8.0
						62	grain	<0.01	<0.01	<0.01	<0.03	8-OH-bentazone				
						62	rest plant[5]	<0.01	<0.01	<0.01	*<0.03*	whole plant[5]	0.01-1.0	6	92.5	5.7
						105	grain	<0.01	<0.01	<0.01	<u><0.03</u>	cobs with husks	0.01-1.0	3	85.5	3.9
						105	rest plant[5]	0.03	0.25	<0.01	0.29	grain	0.01-1.0	3	92.1	3.5
												rest of plant with husks[5]	0.01-1.0	3	88.3	14.8
												rest of plant[5]	0.01-1.0	3	98.9	14.4

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as parent equivalent, the conversion factor for both metabolites is 0.938;
3) expressed as parent equivalent; the conversion factor for both metabolites is 0.938;
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
   for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) at application

_ underlined values were used for MRL calculation for maize
values in **bold** were used for MRL calculation for sweet corn
values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3/106 Residues of bentazone in maize after one application of BAS 351 40 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	347929	Maize	France	BAS 351 40 H: 1 x 1.2	55	0	plant[5]	5.58	2.03	0.29	7.90	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2009/1024805					28	cobs w/o husks	<0.01	0.02	<0.01	**0.04**	Bentazone				
Trial No.	L080406					28	rest w. husks	0.02	2.63	<0.01	2.66	whole plant[5]	0.01-1.0	6	87.0	6.7
GLP:	yes					28	plant[5]	<0.01	1.78	<0.01	1.80	cobs with husks	0.01-1.0	3	87.2	6.2
Year	2008					64	grain	<0.01	<0.01	<0.01	<0.03	grain	0.01-1.0	3	88.5	7.8
						64	rest plant[5]	0.02	1.26	0.01	*1.29*	rest of plant with husks[5]	0.01-1.0	3	87.5	1.4
						76	grain	<0.01	<0.01	<0.01	<0.03	rest of plant[5]	0.01-1.0	3	93.4	2.4
						76	rest plant[5]	0.04	1.03	<0.01	1.08	6-OH-bentazone				

Table 6.3/106 Residues of bentazone in maize after one application of BAS 351 40 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	347929	Maize	Spain	BAS 351 40 H: 1 x 1.2	55	0	plant[5]	0.03	0.025	<0.01	0.065	whole plant[5]	0.01-1.0	6	99.9	6.2
Doc ID:	2009/1024805					28	cobs w/o husks	<0.01	0.08	<0.01	**0.10**	cobs with husks	0.01-1.0	3	104.1	7.8
Trial No.	L080407					28	rest w. husks	0.01	2.36	<0.01	2.38	grain	0.01-1.0	3	109.8	7.9
GLP:	yes					28	plant[5]	<0.01	0.665	<0.01	0.69	rest of plant with husks[5]	0.01-1.0	3	98.1	4.4
Year	2008					63	grain	<0.01	<0.01	<0.01	<0.03	rest of plant[5]	0.01-1.0	3	101.7	8.0
						63	rest plant[5]	<0.01	0.37	<0.01	0.39	8-OH-bentazone				
												whole plant[5]	0.01-1.0	6	92.5	5.7
												cobs with husks	0.01-1.0	3	85.5	3.9
												grain	0.01-1.0	3	92.1	3.5
												rest of plant with husks[5]	0.01-1.0	3	88.3	14.8
												rest of plant[5]	0.01-1.0	3	98.9	14.4

0) actual application rates varied by 10% at most

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
   for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) at application

_ underlined values were used for MRL calculation for maize
values in **bold** were used for MRL calculation for sweet corn
values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Report:	II A 6.3.8/3 Oxspring S. 2008(f) Study on the residue behaviour of Bentazone in corn (maize) and sweet corn after treatment with BAS 351 40 H under field conditions in Northern and Southern Europe during 2007 BASF DocID 2008/1049973
Guidelines:	None
GLP:	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Report:	II A 6.3.8/4 Oxspring S. 2008(e) Final report amendment number 1: Study on the residue behaviour of Bentazone in corn (maize) and sweet corn after treatment with BAS 351 40 H under field conditions in Northern and Southern Europe during 2007 BASF DocID 2008/1055036
Guidelines:	None
GLP:	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

During the 2007 growing season, 6 field trials were conducted in representative maize growing areas in Northern and Southern France, to determine the residue level of bentazone (BAS 351 H) and its metabolites BH 351-6-OH and BH 351-8-OH in or on raw agricultural commodities (RAC).
Therefore BAS 351 40 H, a SL formulation of bentazone (480 g/L BAS 351 H) was applied once at an application rate of 2.5 L/ha corresponding to an application rate of 1.2 kg a.s./ha at crop growth BBCH 55-61. Specimens of whole plant were collected immediately after the application had dried (0 DALA) from each plot as well as cobs without husks and rest of plant with husk (no roots) that were sampled 28-29 days thereafter. At crop growth stage BBCH 73-75 and BBCH 83-85 specimens of cobs without husks and rest of plant with husk (no roots) were also taken with the exception of one trial where the crop was mature enough to sample grain and rest of plant (no roots). At normal commercial harvest (BBCH 89), grain and rest of plant (no roots) were sampled at all maize trials. However due to extremely wet weather conditions in the UK, at Trials AF/12153/BAl3 (L070787) and AF/12153/BA/4 (L070789) whole plant specimens were taken at crop growth stage BBCH 83-85 and allowed to dry in a contamination free glass house for 26 and 28 days respectively until the crop reached BBCH 89.
The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone with BASF Method No. 438/2 (L0044/02) which quantifies the relevant residues with a limit of quantitation (LOQ) of 0.01 mg/kg.
The study showed that residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were only in traces detectable and the total bentazone residues (expressed as bentazone equivalents) ranged between <0.03-0.07 mg/kg on each sampling occasion in grain specimens.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 351 40 H (SL)
Lot/Batch #: 12664
Purity: 480 g/L BAS 351 H nominal
CAS#: 25057-89-0
Development code:
Spiking levels: 0.01-20.0 mg/kg (bentazone, 6-OH-bentazone, 8-OH-bentazone)

2. Test Commodity:

Crop: Maize
Type: Cereals
Variety: Anjou 285, Toccata&Sapphire, Salgado, Eleonora, P33N44, DKC 5784
Botanical name: *Zea mays*
Crop parts(s) or processed commodity: Whole plant without roots, cobs with husks, rest of plant with husks (no roots), grain, rest of plant (no roots)
Sample size: 1 kg/ 12 units

B. STUDY DESIGN

1. Test procedure

A total number of 6 supervised field trials were conducted in representative maize growing areas in Northern France, United Kingdom, Italy and Spain in 2007 to determine the residue level of bentazone and its metabolites BH 351-6-OH and BH 351-8-OH in or on raw agricultural commodities (RAC). Therefore BAS 351 40 H, a SL formulation of bentazone (480 g/L BAS 351 H) was applied once at an application rate of 2.5 L/ha corresponding to an application rate of 1.2 kg a.s./ha at crop growth BBCH 55-61. The spray volume was 200 L/ha. Specimens of whole plant were collected immediately after the application had dried (0 DALA) from each plot as well as cobs without husks and rest of plant with husk (no roots) that were sampled 28-29 days thereafter. At crop growth stage BBCH 73-75 and BBCH 83-85 specimens of cobs without husks and rest of plant with husk (no roots) were also taken with the exception of one trial where the crop was mature enough to sample grain and rest of plant (no roots). At normal commercial harvest (BBCH 89) grain and rest of plant (no roots) were sampled from both plots at all maize trials. However due to extremely wet weather conditions in the UK, at trials AF/12153/BAI3 (L070787) and AF/12153/BA/4 (L070789) whole plant specimens were taken at crop growth stage BBCH 83-85 and allowed to dry in a contamination free glass house for 26 and 28 days respectively until the crop reached BBCH 89.

Table 6.3/107 Target application rates and timings for maize

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2007	6	1	F	BAS 351 40 H (SL)	BAS 351 H	1.2	200	BBCH 55

2. Description of analytical procedures

Residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were determined following BASF Method No. 438/2. Bentazone and its metabolites were extracted from plant matrices with aqueous methanol. The two relevant metabolites which are present as glucosides were hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic plant constituents the extract was filtered and one aliquot taken, evaporated to dryness and dissolved in a methanol/water solution. The final determination of the residues of bentazone and its hydroxyl metabolites was performed by LC-MS/MS. The limit of quantitation (LOQ) of the method was 0.01 mg/kg for each analyte.

II. RESULTS AND DISCUSSION

The residue ranges of bentazone, 6-OH-bentazone and 8-OH-bentazone in maize treated once with the formulation BAS 351 40 H are shown in Table 6.3/108. Details are presented in Table 6.3/109 and Table 6.3/110.

The measured residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone in maize treated once with BAS 351 40 H were below or near the limit of quantitation of 0.01 mg/kg in each analysed cobs with husks and grain sample. As a result, the total residues of bentazone (expressed as bentazone equivalents) were <0.03-0.04 mg/kg on each sampling occasion in grain specimens and <0.03-0.19 mg/kg in cobs with husks specimens. Total bentazone residues in whole plant specimens at 0 DALA ranged between 6.72-13.69 mg/kg. In rest of plant specimens at BBCH 89, total bentazone residues ranged between 0.18-3.22 mg/kg.

No residues of bentazone or its metabolites were found in the untreated control samples except from one rest of plant (no roots) specimen (6-OH-bentazone 0.03 mg/kg).

Table 6.3/108 Summary of residues in maize

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	2007	0	55-61	whole plant[5]	6.15-11.95	0.39-0.99	0.02-0.86	6.72-13.69
		24-29	63-75	cobs w/o husks	<0.01	0.01-0.19	<0.01	0.03-0.19
		36-48	73-85	cobs w/o husks	<0.01	0.01-0.09	<0.01	0.03-0.10
		55-61	83-85	cobs w/o husks	<0.01	0.07-0.08	<0.01	0.09-0.10
		24-29	63-75	rest w husks	0.05-0.13	0.46-1.75	0.03-0.07	0.52-1.78
		36-48	73-85	rest w husks	0.01-0.03	1.02-1.61	0.03-0.08	1.0-1.61
		55-61	83-85	rest w husks	0.02-0.04	1.09-1.79	0.06-0.07	1.11-1.78
		69-89	**89**	**grain**	**<0.01**	**<0.01-0.02**	**<0.01**	**0.03-0.04**
		69-89	89	rest of plant[5]	0.03-0.08	0.44-3.30	0.02-0.07	0.46-3.22
EU South		0	55	whole plant[5]	3.80-5.37	2.58-4.74	0.17-0.32	6.94-10.12
		28	65-73	cobs w/o husks	<0.01	<0.01-0.03	<0.01	<0.03-0.05
		35	73-75	cobs w/o husks	<0.01	<0.01-0.01	<0.01	<0.03-0.03
		42	83-85	cobs w/o husks	<0.01	<0.01	<0.01	<0.03
		28	65-73	rest w husks	0.02-0.33	0.28-0.38	0.02-0.03	0.33-0.71
		35	73-75	rest w husks	0.06-0.29	0.96-1.32	0.04-0.08	1.04-1.56
		42	83-85	rest w husks	0.02-0.13	0.24-0.58	0.01-0.02	0.26-0.69
		43	73-75	grain	<0.01	<0.01	<0.01	<0.03
		50	83-85	grain	<0.01	<0.01	<0.01	<0.03
		65-96	**89**	**grain**	**<0.01**	**<0.01**	**<0.01**	**<0.03**
		43	73-75	rest of plant[5]	0.01	0.50	0.03	0.51
		50	83-85	rest of plant[5]	0.02	0.57	0.02	0.57
		65-96	89	rest of plant[5]	0.01-0.24	0.13-0.44	<0.01-0.01	0.18-0.51

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) without roots

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

The study showed that residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were only in traces detectable and the total bentazone residues (expressed as bentazone equivalents) ranged between <0.03-0.04 mg/kg on each sampling occasion in grain specimens.

Table 6.3/109 Residues of bentazone in maize after one application of BAS 351 40 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Benta-zone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	303258					0	whole plant[5]	6.15	0.59	0.02	6.72	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2008/1055036					24	cobs w/o husk	<0.01	0.02	<0.01	0.04	Bentazone				
	2008/1049973					24	rest of plant[6]	0.05	0.46	0.04	0.52	cob / grain	0.01-1.0	9	88	15
Trial No.	L070788					28	cobs w/o husk	<0.01	0.01	<0.01	**0.03**	rest of pl.	0.01-20.0	26	91	10
GLP:	yes	Maize	France	BAS 351 40 H: 1 x 1.2	55	28	rest of plant[6]	0.07	1.75	0.07	1.78	6-OH-bentazone				
Year	2007					48	cobs w/o husk	<0.01	0.01	<0.01	0.03	cob / grain	0.01-1.0	12	90	11
						48	rest of plant[6]	0.03	1.61	0.08	*1.61*	rest of pl.	0.01-20.0	25	83	8
						69	grain	<0.01	<0.01	<0.01	<u><0.03</u>	8-OH-bentazone				
						69	rest of plant[5]	0.03	0.44	0.02	0.46	cob / grain	0.01-1.0	8	83	8
Study code:	303258					0	whole plant[5]	11.95	0.99	0.86	13.69	rest of pl.	0.01-20.0	28	88	12
Doc ID:	2008/1055036					28	cobs w/o husk	<0.01	0.08	<0.01	**0.10**					
	2008/1049973					28	rest of plant[6]	0.13	1.27	0.05	1.37					
Trial No.	L070787					36	cobs w/o husk	<0.01	0.08	<0.01	0.10					
GLP:	yes	Maize	UK	BAS 351 40 H: 1 x 1.2	55-59	36	rest of plant[6]	0.03	1.21	0.03	1.19					
Year	2007					55	cobs w/o husk	<0.01	0.08	<0.01	0.10					
						55	rest of plant[6]	0.04	1.79	0.06	*1.78*					
						81	grain	<0.01	0.02	<0.01	<u>0.04</u>					
						81	rest of plant[5]	0.06	3.30	0.07	3.22					
Study code:	303258					0	whole plant[5]	9.80	0.39	0.56	10.70					
Doc ID:	2008/1055036					29	cobs w/o husk	<0.01	0.19	<0.01	**0.20**					
	2008/1049973					29	rest of plant[6]	0.06	0.61	0.03	0.66					
Trial No.	L070789					44	cobs w/o husk	<0.01	0.09	<0.01	0.10					
GLP:	yes	Maize	UK	BAS 351 40 H: 1 x 1.2	55-61	44	rest of plant[6]	0.01	1.02	0.04	1.00					
Year	2007					61	cobs w/o husk	<0.01	0.07	<0.01	0.09					
						61	rest of plant[6]	0.02	1.09	0.07	*1.11*					
						89	grain	<0.01	0.02	<0.01	<u>0.04</u>					
						89	rest of plant[5]	0.080	2.13	0.06	2.14					

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
   for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) with husk (no roots)

_ underlined values were used for MRL calculation for maize
values in **bold** were used for MRL calculation for sweet corn
values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.3/110 Residues of bentazone in maize after one application of BAS 351 40 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Benta-zone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	303258	Maize	Italy	BAS 351 40 H: 1 x 1.2	55	0	whole plant[5]	3.80	3.34	0.17	7.09	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2008/1055036					28	cobs w/o husk	<0.01	<0.01	<0.01	**<0.03**	Bentazone				
	2008/1049973					28	rest of plant[6]	0.02	0.30	0.03	0.33	cob / grain	0.01-1.0	9	88	15
Trial No.	L070790					43	grain	<0.01	<0.01	<0.01	<0.03	rest of pl.	0.01-20.0	26	91	10
GLP:	yes					43	rest of plant[5]	0.01	0.50	0.03	0.51	6-OH-bentazone				
Year	2007					50	grain	<0.01	<0.01	<0.01	<0.03	cob / grain	0.01-1.0	12	90	11
						50	rest of plant[5]	0.02	0.57	0.02	*0.57*	rest of pl.	0.01-20.0	25	83	8
						65	grain	<0.01	<0.01	<0.01	<u>0.03</u>	8-OH-bentazone				
						65	rest of plant[5]	0.05	0.13	<0.01	0.18	cob / grain	0.01-1.0	8	83	8
Study code:	303258	Maize	Spain	BAS 351 40 H: 1 x 1.2	55	0	whole plant[5]	4.26	2.58	0.28	6.94	rest of pl.	0.01-20.0	28	88	12
Doc ID:	2008/1055036					28	cobs w/o husk	<0.01	0.03	<0.01	**0.05**					
	2008/1049973					28	rest of plant[6]	0.33	0.38	0.02	0.71					
Trial No.	L070791					35	cobs w/o husk	<0.01	0.01	<0.01	0.03					
GLP:	yes					35	rest of plant[6]	0.29	1.32	0.04	1.56					
Year	2007					42	cobs w/o husk	<0.01	<0.01	<0.01	<0.03					
						42	rest of plant[6]	0.13	0.58	0.02	*0.69*					
						86	grain	<0.01	<0.01	<0.01	<u>0.03</u>					
						86	rest of plant[5]	0.24	0.28	0.01	0.51					
Study code:	303258	Maize	Spain	BAS 351 40 H: 1 x 1.2	55	0	whole plant[5]	5.37	4.74	0.32	10.12					
Doc ID:	2008/1055036					28	cobs w/o husk	<0.01	<0.01	<0.01	**<0.03**					
	2008/1049973					28	rest of plant[6]	0.09	0.28	0.02	0.37					
Trial No.	L070792					35	cobs w/o husk	<0.01	<0.01	<0.01	<0.03					
GLP:	yes					35	rest of plant[6]	0.06	0.96	0.08	1.04					
Year	2007					42	cobs w/o husk	<0.01	<0.01	<0.01	<0.03					
						42	rest of plant[6]	0.02	0.24	0.01	*0.26*					
						96	grain	<0.01	<0.01	<0.01	<u>0.03</u>					
						96	rest of plant[5]	0.01	0.44	<0.01	0.43					

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
   for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) with husk (no roots)

_ underlined values were used for MRL calculation for maize
values in **bold** were used for MRL calculation for sweet corn
values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Report:	II A 6.3.8/5 Reichert N. 2006(d) Study on the residue behaviour of Bentazone and Terbuthylazine in corn after treatment with BAS 600 00 H under field conditions in Germany and the Netherlands, 2005 BASF DocID 2005/1034455
Guidelines:	EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8); EEC 1607/VI/97 rev. 2 10.06.1999; EEC 7029/VI/95 rev. 5; EEC 7525/VI/95 rev. 7; SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	Yes (laboratory certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Report:	II A 6.3.8/6 Reichert N. 2006(c) 1 Amendment: Study on the residue behaviour of Bentazone and Terbuthylazine in corn after treatment with BAS 600 00 H under field conditions in Germany and the Netherlands, 2005 BASF DocID 2006/1024264
Guidelines:	EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8); EEC 1607/VI/97 rev. 2 10.06.1999; EEC 7029/VI/95 rev. 5; EEC 7525/VI/95 rev. 7; SANCO/3029/99 rev. 4 (11 July 2000)
GLP:	Yes (laboratory certified by Hessisches Ministerium fuer Umwelt, Laendlichen Raum und Verbraucherschutz)

Executive Summary

In 2005 two field trials were conducted in two representative maize growing areas in Northern Europe (the Netherlands and Germany). The herbicidal product BAS 600 00 H (200 g/L BAS 351 H, SC) was applied once at an application rate of 4 L/ha corresponding to an application rate of 0.8 kg a.s./ha. Only the residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone are reported here. The application took place at GS 14-15 with a spray rate of 300 L/ha. Specimens were taken directly after the treatment, at BBCH 34-38, BBCH 85 and BBCH 89. Specimens were analysed by means of BASF Method No. 438/1. The limit of quantitation was 0.02 mg/kg for all analysed substances. No quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable and the total bentazone residues (expressed as bentazone equivalents) were below 0.06 mg/kg on each grain sampling occasion.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 600 00 H (SC)
Lot/Batch #: 1132
Purity: 200 g/L BAS 351 H; 200 g/L Terbuthylazine
CAS#: 25057-89-0 (bentazone)
Development code:
Spiking levels: 0.02 and 100 mg/kg (bentazone, 6-OH-bentazone, 8-OH-bentazone)

2. Test Commodity:

Crop: Maize
Type: Cereals
Variety: Ohio, HSMR 20
Botanical name: *Zea mays*
Crop parts(s) or processed commodity: Whole plant without roots, cobs with husks, rest of plant without roots and grains
Sample size: 0.2-2 kg

B. STUDY DESIGN

1. Test procedure 2 field trials were conducted in two representative maize growing areas in the Netherlands and Germany in 2005. The herbicidal product BAS 600 00 H (200 g/L BAS 351 H, SC) was applied once at an application rate of 4 L/ha corresponding to an application rate of 0.8 kg a.s./ha. The application took place at BBCH 14-15 with a spray rate of 300 L/ha. Specimens were taken directly after the treatment, at BBCH 34-38, BBCH 85 and BBCH 89. Collected samples were stored frozen at -18°C until further analysis. The maximum storage interval for plant matrices from harvest until extraction was 323 days.

Table 6.3/111 Target application rates and timings for maize

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2005	2	1	F	BAS 600 00 H (SC)	BAS 351 H	0.8	300	BBCH 12-15

2. Description of analytical procedures

Residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were determined following BASF Method No. 438/1. Bentazone and its metabolites are extracted from plant matrices with aqueous methanol. After purification by an iso-octane liquid-liquid partition, the two relevant metabolites which are present as glucosides are hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic plant constituents, a reversed phase C_{18}-SPE clean-up is performed. The final determination of bentazone and its hydroxyl-metabolites was performed by HPLC-MS/MS, monitoring two parent-daughter ion transitions for each compound. The method achieves a limit of quantitation (LOQ) of 0.02 mg/kg.

II. RESULTS AND DISCUSSION

The residue ranges of bentazone, 6-OH-bentazone and 8-OH-bentazone in maize treated once with the single formulation BAS 600 00 H are shown in Table 6.3/112. Details can be found in Table 6.3/113.

The residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone measured in maize whole plant without root specimens treated once with BAS 600 00 H ranged between 79.5-133.9 mg/kg and were below the limit of quantitation of <0.02 mg/kg in all other analysed maize samples. As a result, the total residues of bentazone (expressed as bentazone equivalents) were below 0.06 mg/kg on each sampling occasion.

No residues of bentazone or its metabolites were found in the untreated control samples.

Table 6.3/112 Summary of residues in maize

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	2005	0	14-15	plant[5]	78-128	1.3-6	0.3-0.32	79.5-133.9
		28-36	34-38	plant[5]	<0.02	<0.02	<0.02	<0.06
		103-117	85	cob with husks	<0.02	<0.02	<0.02	<0.06
		103-117	85	rest of plant[5]	<0.02	<0.02	<0.02	<0.06
		138-142	**89**	**grains**	**<0.02**	**<0.02**	**<0.02**	**<0.06**
		138-142	89	rest of plant[5]	<0.02	<0.02	<0.02	<0.06

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) without roots

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

No quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable and the total bentazone residues (expressed as bentazone equivalents) were below 0.06 mg/kg on each sampling occasion and each specimen except whole plant without root specimens at 0 DALA.

Table 6.3/113 Residues of bentazone in maize after one application of BAS 600 00 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	227041	Maize	The Netherlands	BAS 600 00 H: 1 x 0.8	14	0	plant[5]	128	6	0.32	133.9	**Method:** No. 438/1; LOQ = 0.02 mg/kg				
Doc ID:	2005/1034455					28	plant[5]	<0.02	<0.02	<0.02	<0.06	Bentazone*				
Trial No.	AGR/22/05					103	cobs with husks	<0.02	<0.02	<0.02	<0.06	cobs/grain	0.02-0.2	10	94.9	12.2
GLP:	yes					103	rest of plant[5]	<0.02	<0.02	<0.02	*<0.06*	plant[5]	0.02-100	13	104.5	16.7
Year	2005					138	grains	<0.02	<0.02	<0.02	<u>≤0.06</u>	6-OH-bentazone**				
						138	rest of plant[5]	<0.02	<0.02	<0.02	<0.06	cobs/grain	0.02-0.2	10	67.7	19.0
Study code:	227041	Maize	Germany	BAS 600 00 H: 1 x 0.8	15	0	plant[5]	78	1.3	0.3	79.5	plant[5]	0.02-100	11	81.6	19.9
Doc ID:	2005/1034455					36	plant[5]	<0.02	<0.02	<0.02	<0.06	8-OH-bentazone***				
Trial No.	AGR/23/05					117	cobs with husks	<0.02	<0.02	<0.02	<0.06	cobs/grain	0.02-0.2	10	69.7	7.0
GLP:	yes					117	rest of plant[5]	<0.02	<0.02	<0.02	*<0.06*	plant[5]	0.02-100	11	85.4	22.9
Year	2005					142	grains	<0.02	<0.02	<0.02	<u>≤0.06</u>					
						142	rest of plant[5]	<0.02	<0.02	<0.02	<0.06					

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938; for calculation purposes, residues were treated as follows: <0.02 + <0.02 + <0.02 = <0.06
5) without roots
6) at application

_ underlined values were used for MRL calculation

* transition 239 m/z → 132 m/z

** transition 255 m/z → 213 m/z

*** transition 255 m/z → 191 m/z

values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Report:	II A 6.3.8/7 Schulz H. 2001(b) Determination of the residues of Reg.No. 271 272 and Bentazone in maize following treatment with BAS 635 00 H, BAS 351 40 H and BAS 152 00 S under field conditions in France 1999 BASF DocID 2001/1000919
Guidelines:	BBA IV 3-3; FAO Guidelines Rome 1990; IVA Guidelines for Residue Studies Sections IA and IB 2nd edition 1992
GLP:	Yes (laboratory certified by Hessisches Ministerium fuer Umwelt, Landwirtschaft und Forsten, Wiesbaden)

Executive Summary

A total number of four field trials were conducted in 4 representative maize growing areas in Northern and Southern France during 1999. The herbicidal product BAS 351 40 H (480 g/L BAS 351 H, SL) was nominally applied (in concert with the formulation BAS 635 00 H and the adjuvant BAS 152 00 S) at an application rate of 3.2 L/ha corresponding to an application rate of 1.54 kg a.s./ha. The first samples (whole plants) were collected 14-16 and 34-51 days after the last application and subsequent samples were taken on day 85 (cobs with husks) and 89 days (grain, straw) post application. Specimens were analysed by means of BASF Method No. 438/1 (which is a miniaturised version of BASF Method No. 438/0). The limit of quantification was 0.02 mg/kg for all analysed substances. The study showed that no quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable in cobs (with husks), grain and straw samples. Therefore the total bentazone residues (expressed as bentazone equivalents) were below 0.06 mg/kg in all investigated samples. Therefore, the residue values were used for MRL determination (see chapter 6.7) even though an exaggerated application rate compared to the critical GAP was used.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description: BAS 351 40 H (SL)
Lot/Batch #: 99-1
Purity: 480 g/L BAS 351 H
CAS#: 25057-89-0
Development code:
Spiking levels: 0.02-2.0 mg/kg (Bentazone, 6-OH-bentazone, 8-OH-bentazone)

2. Test Commodity:

Crop: Maize
Type: Cereals
Variety: Chantal, Anjou 258, LG 2447, DK 604
Botanical name: *Zea mays*
Crop parts(s) or processed commodity: Whole plant, cobs with husks, rest plants without roots, grain, straw
Sample size: 0.30-12 kg

B. STUDY DESIGN

1. Test procedure

A total number of four field trials were conducted in 4 representative maize growing areas in Northern and Southern France during 1999. The herbicidal product BAS 351 40 H (480 g/L BAS 351 H, SL) was nominally applied (in concert with the formulation BAS 635 00 H and the adjuvant BAS 152 00 S) at an application rate of 3.2 L/ha corresponding to an application rate of 1.54 kg a.s./ha at BBCH 14-16. The first samples (whole plants) were collected 14-16 and 34-51 days after the last application and subsequent samples were taken on day 85 cobs with husks) and 89 days (grain, straw) post application. Collected samples were stored frozen at -20°C until further analysis.

Table 6.3/114 Target application rates and timings for maize

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
1999	4	1	F	BAS 351 40 H (SL)	BAS 351 H	1.54	250	BBCH 14-16

2. Description of analytical procedures

Residues of bentazone, 6-OH-bentazone, 8-OH-bentazone were determined following BASF Method No. 438/1 (which is a miniaturised version of BASF Method No. 438/0). Bentazone and its metabolites are extracted from plant matrices with aqueous methanol. After purification by an iso-octane liquid-liquid partition, the two relevant metabolites which are present as glucosides are hydrolyzed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic plant constituents, a reversed phase C_{18}-column clean-up is performed. The analytes are then methylated with diazomethane and their derivatives are purified using a silica gel-column. The final determination of the residues of bentazone and its hydroxy metabolites is performed by GC/MS. The limit of quantification was 0.02 mg/kg for all analysed substances.

II. RESULTS AND DISCUSSION

The residue ranges of bentazone, 6-OH-bentazone and 8-OH-bentazone in maize commodities treated once with the formulation BAS 351 40 F are shown in Table 6.3/115. Details can be found in Table 6.3/116 and Table 6.3/117.

Highest residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were measured in whole plants collected directly after the last application (total bentazone: 80.73-170.0 mg/kg), whilst residues were considerably lower in whole plant specimens 30 to 45 days after treatment (0.18-0.26 mg/kg total bentazone). No quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable in any of the investigated cob (with husks), grain and straw samples. As a result, the total residues of bentazone (expressed as bentazone equivalents) in cobs (with husks), grain and straw samples were below 0.06 mg/kg in each sample.

Table 6.3/115 Summary of residues in maize

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	1999	0	14-16	plant[5]	99.94-166.3	1.25-3.53	0.13-0.419	101.23-170.0
		30-45	34-51	plant[5]	0.128-0.133	0.027-0.12	<0.02	0.18-0.26
		76-115	85	cob with husks	<0.02	<0.02	<0.02	<0.06
		76-115	85	rest of plant[5]	<0.02	<0.02	<0.02	<0.06
		107-140	**89**	**grains**	**<0.02**	**<0.02**	**<0.02**	**<0.06**
		107-140	89	straw	<0.02	<0.02	<0.02	<0.06
EU South	1999	0	15-19	plant[5]	78.84-155.6	1.82-6.14	0.2-0.378	80.73-161.71
		46-48	35-51	plant[5]	0.371-0.833	<0.02	0.022	0.41-0.87
		108-111	85	cob with husks	<0.02	<0.02	<0.02	<0.06
		108-111	85	rest of plant[5]	<0.02	<0.02	<0.02	<0.06
		141-144	**89**	**grains**	**<0.02**	**<0.02**	**<0.02**	**<0.06**
		141-144	89	straw	<0.02	<0.02	<0.02	<0.06

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
5) without roots

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

The study showed that no quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable in cobs (with husks), grain and straw samples and therefore the total bentazone residues (expressed as bentazone equivalents) were below 0.06 mg/kg in all investigated samples.

Table 6.3/116 Residues of bentazone in maize after one application of BAS 351 45 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	IF-99/09969-00	Maize	France	BAS 351 45 H: 1 x 1.54	14-16	0	plant[5]	166.3	3.53	0.419	170.0	**Method:** No. 438/1; LOQ = 0.02 mg/kg				
Doc ID:	2001/1000919					45	plant[5]	0.133	0.027	<0.02	0.18	Bentazone				
Trial No.	X996220					115	cobs with husks	<0.02	<0.02	<0.02	<0.06	w. plant[5]	0.02-2.0	2	83.5	N/A
GLP:	yes					115	rest of plant[5]	<0.02	<0.02	<0.02	*<0.06*	cobs with husks	0.02-0.2	2	98.9	N/A
Year	1999					140	grain	<0.02	<0.02	<0.02	<0.06	rest of plant[5]	0.02-0.2	2	93	N/A
						140	straw	<0.02	<0.02	<0.02	<0.06	grain	0.02-0.2	2	100.2	N/A
Study code:	IF-99/09969-00	Maize	France	BAS 351 45 H: 1 x 1.54	15-16	0	plant[5]	99.94	1.25	0.13	101.23	straw	0.02-0.2	2	93.4	N/A
Doc ID:	2001/1000919					30	plant[5]	0.128	0.12	<0.02	0.26	6-OH-bentazone				
Trial No.	X996221					76	cobs with husks	<0.02	<0.02	<0.02	<0.06	w. plant[5]	0.02-2.0	2	82.1	N/A
GLP:	yes					76	rest of plant[5]	<0.02	<0.02	<0.02	*<0.06*	cobs with husks	0.02-0.2	2	81.8	N/A
Year	1999					107	grain	<0.02	<0.02	<0.02	<0.06	rest of plant[5]	0.02-0.2	2	79.5	N/A
						107	straw	<0.02	<0.02	<0.02	<0.06	grain	0.02-0.2	2	88.1	N/A
												straw	0.02-0.2	2	87.3	N/A
												8-OH-bentazone				
												w. plant[5]	0.02-2.0	2	83.4	N/A
												cobs with husks	0.02-0.2	2	94.6	N/A
												rest of plant[5]	0.02-0.2	2	80.3	N/A
												grain	0.02-0.2	2	89.7	N/A
												straw	0.02-0.2	2	90.4	N/A

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
   for calculation purposes, residues were treated as follows: <0.02 + <0.02 + <0.02 = <0.06
5) without roots
6) at application
_ underlined values were used for MRL calculation
N/A not applicable

Table 6.3/117 Residues of bentazone in maize after one application of BAS 351 45 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	IF-99/09969-00	Maize	France	BAS 351 45 H: 1 x 1.54	15	0	plant[5]	155.6	6.14	0.378	161.71	**Method:** No. 438/1; LOQ = 0.02 mg/kg				
Doc ID:	2001/1000919					46	plant[5]	0.833	<0.02	0.022	0.87	Bentazone				
Trial No.	X996230					111	cobs with husks	<0.02	<0.02	<0.02	<0.06	w. plant[5]	0.02-2.0	2	83.5	N/A
GLP:	yes					111	rest of plant[5]	<0.02	<0.02	<0.02	*<0.06*	cobs with husks	0.02-0.2	2	98.9	N/A
Year	1999					144	grain	<0.02	<0.02	<0.02	<u>≤0.06</u>	rest of plant[5]	0.02-0.2	2	93	N/A
						144	straw	<0.02	<0.02	<0.02	<0.06	grain	0.02-0.2	2	100.2	N/A
Study code:	IF-99/09969-00	Maize	France	BAS 351 45 H: 1 x 1.54	15-16	0	plant[5]	78.84	1.82	0.20	80.73	straw	0.02-0.2	2	93.4	N/A
Doc ID:	2001/1000919					48	plant[5]	0.371	<0.02	0.022	0.41	6-OH-bentazone				
Trial No.	X996231					108	cobs with husks	<0.02	<0.02	<0.02	<0.06	w. plant[5]	0.02-2.0	2	82.1	N/A
GLP:	yes					108	rest of plant[5]	<0.02	<0.02	<0.02	*<0.06*	cobs with husks	0.02-0.2	2	81.8	N/A
Year	1999					141	grain	<0.02	<0.02	<0.02	<u>≤0.06</u>	rest of plant[5]	0.02-0.2	2	79.5	N/A
						141	straw	<0.02	<0.02	<0.02	<0.06	grain	0.02-0.2	2	88.1	N/A
												straw	0.02-0.2	2	87.3	N/A
												8-OH-bentazone				
												w. plant[5]	0.02-2.0	2	83.4	N/A
												cobs with husks	0.02-0.2	2	94.6	N/A
												rest of plant[5]	0.02-0.2	2	80.3	N/A
												grain	0.02-0.2	2	89.7	N/A
												straw	0.02-0.2	2	90.4	N/A

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938; for calculation purposes, residues were treated as follows: <0.02 + <0.02 + <0.02 = <0.06
5) without roots
6) at application

_ underlined values were used for MRL calculation
N/A not applicable
values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Report:	II A 6.3.8/8 Blaschke U.G. 2000(j) Determination of the magnitude of the residues of Basagran (BAS 351 32 H) applied to grain maize in Southern Europe in 1998 BASF DocID 2000/1018489
Guidelines:	EEC 91/414; FAO Guidelines Rome 1990; IVA Guidelines for Residue Studies Sections IA and IB 2nd edition 1992
GLP:	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

A total number of six field trials were conducted in different representative maize growing areas in Spain and Italy during the growing season 1998. The herbicidal product BAS 351 32 H (485 g/L BAS 351 H) was applied at an application rate of 3.2 L/ha corresponding to an application rate of 1.55 kg as/ha. The first samples (shoots and roots) were collected directly after the last application and subsequent samples were taken on day 23-44 (shoots and roots), day 68-97 (ears, shoots and roots) and day 100-127 (grains, straw and roots). Specimens were analysed by means of BASF Method No. 438/1 (which is a miniaturised version of BASF Method No. 438/0). The limit of quantitation was 0.02 mg/kg for all analysed substances. The study showed that in most grain and ear samples no quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable. Therefore the total bentazone residues (expressed as bentazone equivalents) were below 0.06 mg/kg in all grain samples and were at most 0.07 mg/kg in ears. Therefore, the residue values were used for MRL determination (see chapter 6.7) even though an exaggerated application rate compared to the critical GAP was used.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 32 H (SL)
Lot/Batch #:	98-1
Purity:	485 g/L BAS 351 H
CAS#:	25057-89-0
Development code:	
Spiking levels:	0.02-20.0 mg/kg (Bentazone, 6-OH-bentazone, 8-OH-bentazone)

2. Test Commodity:

Crop:	Maize
Type:	Cereals
Variety:	Dracuna, Eleonora, Juanita, Costanza, Alicia.
Botanical name:	*Zea mays*
Crop parts(s) or processed commodity:	Shoots, roots, ears, grain, straw,
Sample size:	0.10-4.8 kg

B. STUDY DESIGN

1. Test procedure

A total number of six field trials were conducted in different representative maize growing areas in Spain and Italy during the growing season 1998. The herbicidal product BAS 351 32 H (485 g/L BAS 351 H) was applied at an application rate of 3.2 L/ha corresponding to an application rate of 1.55 kg as/ha. The first samples (shoots and roots) were collected directly after the last application and subsequent samples were taken on day 23-44 (shoots and roots), day 68-97 (ears, shoots and roots) and day 100-127 (grains, straw and roots). Collected samples were stored frozen at -20°C until further analysis.

Table 6.3/118 Target application rates and timings for maize

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
1998	6	1	F	BAS 351 32 H (SL)	BAS 351 H	1.55	300	BBCH 13-15

2. Description of analytical procedures

Residues of bentazone, 6-OH-bentazone, 8-OH-bentazone were determined following BASF Method No. 438/1 (which is a miniaturised version of BASF Method No. 438/0). Bentazone and its metabolites are extracted from plant matrices with aqueous methanol. After purification by an iso-octane liquid-liquid partition, the two relevant metabolites which are present as glucosides are hydrolyzed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic plant constituents, a reversed phase C_{18}-column clean-up is performed. The analytes are then methylated with diazomethane and their derivatives are purified using a silica gel-column. The final determination of the residues of bentazone and its hydroxy metabolites is performed by GC/MS. The limit of quantification was 0.02 mg/kg for all analysed substances.

II. RESULTS AND DISCUSSION

The residue ranges of bentazone, 6-OH-bentazone and 8-OH-bentazone in maize commodities treated once with the formulation BAS 351 32 F are shown in Table 6.3/119. Details can be found in Table 6.3/120.
The residues of bentazone, 6-OH-bentazone and 8-OH-bentazone in grains and ears were generally below the limit of quantitation or slightly above, respectively. This resulted in total bentazone levels (calculated as bentazone equivalents) below 0.06 mg/kg in all grain samples and reaching at most 0.07 mg/kg in ears. Highest residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were measured in shoots and straw with total residues of bentazone (expressed as bentazone equivalents) ranging from <0.06-106.84 mg/kg and from 0.06-0.23 mg/kg, respectively.

Table 6.3/119 Summary of residues in maize

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU South	1998	0	13-15	shoots*	46.54-103.09	1.88-3.44	0.575-2.10	49.87-106.84
		23-44	39-51	shoots*	0.064-0.411	<0.02-0.183	<0.02-0.038	0.11-0.49
		68-97	83-85	shoots***	<0.02	<0.02-0.034	<0.02	<0.06-0.07
		0	13-15	roots	0.086-2.67	0.028-0.67	<0.02-0.061	0.14-3.36
		23-44	39-51	roots	<0.02	0.031-0.238	<0.02	0.07-0.26
		68-97	83-85	roots	<0.02-0.022	<0.02-0.217	<0.02	<0.06-0.24
		100-127	89	roots	<0.02-0.038	<0.02-0.192	<0.02-0.049	0.06-0.26
		68-97	83-85	ears**	<0.02-0.033	<0.02	<0.02	<0.06-0.07
		100-127	**89**	**grain**	**<0.02**	**<0.02**	**<0.02**	**<0.06**
		100-127	89	straw	<0.02-0.142	<0.02-0.038	<0.02-0.058	<0.06-0.23

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
* without roots
** with husks
*** remaining shoots without ears
Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

The study showed that in all grain and most ear samples no quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable. Therefore the total bentazone residues (expressed as bentazone equivalents) were below 0.06 mg/kg in all grain samples and were at most 0.07 mg/kg in ears.

Table 6.3/120 Residues of bentazone in maize after one application of BAS 351 32 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	BSF 593/984868	Maize	Spain	BAS 351 32 H: 1 x 1.55	15	0	shoots[5]	57.5	2.15	1.68	61.09	**Method:** No. 438/1; LOQ = 0.02 mg/kg				
Doc ID:	2000/1018489					0	roots	0.151	0.028	<0.02	0.20	Bentazone				
Trial No.	BSF/593-1					44	shoots	0.105	<0.02	<0.02	0.14	shoots*	0.2-20	2	85.1	N/A
GLP:	yes					44	roots	<0.02	0.078	<0.02	0.11	roots	0.02-2.0	9	88.8	19.5
Year	1998					81	ears*	<0.02	<0.02	<0.02	<0.06	ears**	0.02-0.2	2	71.1	N/A
						81	shoots**	<0.02	<0.02	<0.02	*<0.06*	shoots***	0.02-0.2	2	77.1	N/A
						81	roots	<0.02	0.099	<0.02	0.13	grain	0.02-0.2	2	95.2	N/A
						122	grain	<0.02	<0.02	<0.02	<u><0.06</u>	straw	0.02-0.2	2	79.6	N/A
						122	straw	<0.02	<0.02	<0.02	<0.06	6-OH-bentazone				
						122	roots	<0.02	0.084	<0.02	0.12	shoots*	0.2-20	2	66.1	N/A
Study code:	BSF 593/984868	Maize	Spain	BAS 351 32 H: 1 x 1.55	15	0	shoots[5]	73.30	2.58	2.10	77.69	roots	0.02-2.0	9	80.6	16.0
Doc ID:	2000/1018489					0	roots	0.086	0.038	<0.02	0.14	ears**	0.02-0.2	2	69.9	N/A
Trial No.	BSF/593-2					38	shoots	0.166	<0.02	<0.02	0.20	shoots***	0.02-0.2	2	62.4	N/A
GLP:	yes					38	roots	<0.02	0.238	<0.02	0.26	grain	0.02-0.2	2	90.5	N/A
Year	1998					68	ears*	<0.02	<0.02	<0.02	<0.06	straw	0.02-0.2	2	93.5	N/A
						68	shoots**	<0.02	<0.02	<0.02	*<0.06*	8-OH-bentazone				
						68	roots	<0.02	0.175	<0.02	0.20	shoots*	0.2-20	2	59.4	N/A
						109	grain	<0.02	<0.02	<0.02	<u><0.06</u>	roots	0.02-2.0	9	81.6	26.7
						109	straw	0.142	0.038	0.056	0.23	ears**	0.02-0.2	2	85.8	N/A
						109	roots	0.038	0.192	0.049	0.26	shoots***	0.02-0.2	2	80.5	N/A

Table 6.3/120 Residues of bentazone in maize after one application of BAS 351 32 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	BSF 593/984868	Maize	Spain	BAS 351 32 H: 1 x 1.55	15	0	shoots[5]	57.82	1.88	1.60	61.08	grain	0.02-0.2	2	79.9	N/A
Doc ID:	2000/1018489					0	roots	0.683	0.028	0.021	0.73	straw	0.02-0.2	2	75.7	N/A
Trial No.	BSF/593-3					38	shoots	0.411	0.051	0.038	0.49					
GLP:	yes					38	roots	<0.02	0.058	<0.02	0.09					
Year	1998					68	ears*	0.033	<0.02	<0.02	0.07					
						68	shoots**	<0.02	0.034	<0.02	*0.07*					
						68	roots	0.022	0.217	<0.02	0.24					
						109	grain	<0.02	<0.02	<0.02	<0.06					
						109	straw	0.133	0.034	0.058	0.22					
						109	roots	<0.02	0.099	<0.02	0.13					
Study code:	BSF 593/984868	Maize	Italy	BAS 351 32 H: 1 x 1.55	13-15	0	shoots[5]	72.85	3.44	0.602	76.64					
Doc ID:	2000/1018489					0	roots	0.363	0.162	<0.02	0.53					
Trial No.	BSF/593-4					23	shoots	0.064	0.030	<0.02	0.11					
GLP:	yes					23	roots	<0.02	0.061	<0.02	0.10					
Year	1998					84	ears*	<0.02	<0.02	<0.02	<0.06					
						84	shoots**	<0.02	<0.02	<0.02	*<0.06*					
						84	roots	0.020	0.074	<0.02	0.11					
						100	grain	<0.02	<0.02	<0.02	<0.06					
						100	straw	<0.02	<0.02	<0.02	<0.06					
						100	roots	0.020	0.043	<0.02	0.08					

Table 6.3/120 Residues of bentazone in maize after one application of BAS 351 32 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	BSF 593/984868	Maize	Italy	BAS 351 32 H: 1 x 1.55	13-15	0	shoots[5]	103.09	1.92	2.08	106.84					
Doc ID:	2000/1018489					0	roots	1.14	0.164	0.036	1.33					
Trial No.	BSF/593-5					36	shoots	0.105	0.183	<0.02	0.30					
GLP:	yes					36	roots	<0.02	0.067	<0.02	0.10					
Year	1998					93	ears*	<0.02	<0.02	<0.02	<0.06					
						93	shoots**	<0.02	<0.02	<0.02	*<0.06*					
						93	roots	<0.02	0.033	<0.02	0.07					
						114	grain	<0.02	<0.02	<0.02	<0.06					
						114	straw	<0.02	<0.02	<0.02	<0.06					
						114	roots	<0.02	0.024	<0.02	0.06					
Study code:	BSF 593/984868	Maize	Italy	BAS 351 32 H: 1 x 1.55	13-15	0	shoots[5]	46.54	2.98	0.575	49.87					
Doc ID:	2000/1018489					0	roots	2.67	0.670	0.061	3.36					
Trial No.	BSF/593-6					36	shoots	0.125	<0.02	<0.02	0.16					
GLP:	yes					36	roots	<0.02	0.031	<0.02	0.07					
Year	1998					97	ears*	<0.02	<0.02	<0.02	<0.06					
						97	shoots**	<0.02	<0.02	<0.02	*<0.06*					
						97	roots	<0.02	<0.02	<0.02	<0.06					
						127	grain	<0.02	<0.02	<0.02	<0.06					
						127	straw	<0.02	<0.02	<0.02	<0.06					
						127	roots	0.020	<0.02	<0.02	0.06					

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938; for calculation purposes, residues were treated as follows: <0.02 + <0.02 + <0.02 = <0.06
5) without roots
6) at application

_ underlined values were used for MRL calculation
N/A not applicable
* with husks
** remaining shoots without ears
values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Barley

Report:	II A 6.3.8/9 Blaschke U.G. 2000(k) Determination of the magnitude of the residue of BAS 351 32 H in/on barley raw agricultural commodity specimens from supervised field trials in Southern Europe in 1999 BASF DocID 2000/1018490
Guidelines:	FAO Guidelines Rome 1990; EEC 87/18; EEC 91/414 Annex II (Part A Section 6); EEC 91/414 Annex III (Part A Section 8); EEC 96/68
GLP:	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

A total number of five field trials were conducted at different representative barley growing areas in Southern Europe (Spain and Italy) during 1999. The herbicidal product BAS 351 32 H (485 g/L BAS 351 H) was applied at an application rate of 3.2 L/ha corresponding to an application rate of 1.55 kg a.s./ha. The first samples (shoots) were collected directly after the last application and subsequent samples were taken on day 17-26 (ears, shoots) and 57-65 days (grain, straw). Specimens were analysed by means of BASF Method No. 438/1 (which is a miniaturised version of BASF Method No. 438/0). The limit of quantification was 0.02 mg/kg for all analysed substances. The study showed that no quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable in barley grains and therefore the total bentazone residues (expressed as bentazone equivalents) were below 0.06 mg/kg in all investigated samples. Therefore, the residue values were used for MRL determination (see chapter 6.7) even though an exaggerated application rate compared to the critical GAP was used.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 32 H (SL)
Lot/Batch #:	98-1
Purity:	485 g/L BAS 351 H
CAS#:	25057-89-0
Development code:	
Spiking levels:	0.02-0.2 mg/kg (Bentazone, 6-OH-bentazone, 8-OH-bentazone)

2. Test Commodity:

Crop:	Barley
Type:	Cereals
Variety:	Apex, Gotic, Federal, Folgore, Irene
Botanical name:	*Hordeum vulgare*
Crop parts(s) or processed commodity:	Shoots, ears, grain, straw
Sample size:	0.6-5 kg

B. STUDY DESIGN

1. Test procedure

A total number of five field trials were conducted at different representative barley growing areas in Southern Europe (Spain and Italy) during 1999. The herbicidal product BAS 351 32 H (485 g/L BAS 351 H) was applied at an application rate of 3.2 L/ha corresponding to an application rate of 1.55 kg a.s./ha. The first samples (shoots) were collected directly after the last application and subsequent samples were taken on day 17-26 (ears, shoots) and 57-65 days (grain, straw). Collected samples were stored frozen at -20°C until further analysis.

Table 6.3/121 Target application rates and timings for barley

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
1999	5	1	F	BAS 351 32 H (SL)	BAS 351 H	1.55	300	BBCH 33-39

2. Description of analytical procedures

Residues of bentazone, 6-OH-bentazone, 8-OH-bentazone were determined following BASF Method No. 438/1 (which is a miniaturised version of BASF Method No. 438/0). Bentazone and its metabolites are extracted from plant matrices with aqueous methanol. After purification by an iso-octane liquid-liquid partition, the two relevant metabolites which are present as glucosides are hydrolyzed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic plant constituents, a reversed phase C_{18}-column clean-up is performed. The analytes are then methylated with diazomethane and their derivatives are purified using a silica gel-column. The final determination of the residues of bentazone and its hydroxy metabolites is performed by GC/MS. The limit of quantification was 0.02 mg/kg for all analysed substances.

II. RESULTS AND DISCUSSION

The residue ranges of bentazone, 6-OH-bentazone and 8-OH-bentazone in barley commodities treated once with the formulation BAS 351 32 F are shown in Table 6.3/122. Details are presented in Table 6.3/123.

No quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable in any of the investigated barley grain samples. As a result, the total residues of bentazone (expressed as bentazone equivalents) in grains were below 0.06 mg/kg in any field trial. Highest residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were measured in barley shoots directly after the last application the total bentazone residues (expressed as bentazone equivalents) ranged from 5.15-43.41 mg/kg) and in remaining shoots (PHI 17-26 days) with total bentazone residual levels (expressed as bentazone equivalents) between 0.61 and 4.68 mg/kg. Total bentazone residues levels (expressed as bentazone equivalents) were detected in ears (range from 0.07-0.49 mg/kg) and straw (range from 0.10-0.65 mg/kg).

Table 6.3/122 Summary of residues in barley

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU South	1999	0	33-39	shoots	4.86-40.49	0.278-3.34	0.027-0.252	5.15-43.41
		17-26	61-67	ears	0.020-0.035	0.027-0.461	<0.02-0.030	0.07-0.49
		17-26	61-67	remaining shoots	<0.02-1.03	0.614-4.91	<0.02-0.026	0.61-4.68
		57-65	**89**	**grain**	**<0.02**	**<0.02**	**<0.02**	**<0.06**
		57-65	89	straw	0.039-0.144	0.046-0.633	<0.02	0.10-0.65

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

The study showed that no quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable in barley grains and therefore the total bentazone residues (expressed as bentazone equivalents) were below 0.06 mg/kg in all investigated grain samples.

Table 6.3/123 Residues of bentazone in barley after one application of BAS 351 32 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	BSF 605/002847	Barley	Spain	BAS 351 32 H: 1 x 1.55	33	0	shoots	40.49	2.86	0.252	43.41	**Method:** No. 438/1; LOQ = 0.02 mg/kg				
Doc ID:	2000/1018490					20	ears	0.024	0.263	<0.02	0.29	Bentazone				
Trial No.	BSF/605-1					20	remaining shoots	0.05	4.91	0.026	4.68	remaining shoots	0.02-2.0	2	97.1	N/A
GLP:	yes					58	grains	<0.02	<0.02	<0.02	<0.06	ears	0.02-2.0	2	97	N/A
Year	1999					58	straw	0.039	0.633	<0.02	*0.65*	straw	0.02-2.0	2	95.8	N/A
Study code:	BSF 605/002847	Barley	Spain	BAS 351 32 H: 1 x 1.55	35-37	0	shoots	28.75	3.34	0.217	32.09	shoots	0.02-2.0	2	85.6	N/A
Doc ID:	2000/1018490					17	ears	0.035	0.461	0.029	0.49	grain	0.02-0.2	2	82.2	N/A
Trial No.	BSF/605-2					17	remaining shoots	1.03	3.58	0.022	4.41	6-OH-bentazone				
GLP:	yes					58	grains	<0.02	<0.02	<0.02	<0.06	remaining shoots	0.02-2.0	2	84.8	N/A
Year	1999					58	straw	0.144	0.23	<0.02	*0.38*	ears	0.02-2.0	2	102.4	N/A
Study code:	BSF 605/002847	Barley	Italy	BAS 351 32 H: 1 x 1.55	37	0	shoots	18.13	0.714	0.188	18.98	straw	0.02-2.0	2	91.1	N/A
Doc ID:	2000/1018490					26	ears	0.020	0.027	0.030	0.07	shoots	0.02-2.0	2	90.9	N/A
Trial No.	BSF/605-3					26	remaining shoots	<0.02	0.697	<0.02	0.69	grain	0.02-0.2	2	88.9	N/A
GLP:	yes					65	grains	<0.02	<0.02	<0.02	<0.06	8-OH-bentazone				
Year	1999					65	straw	0.058	0.091	<0.02	*0.16*	remaining shoots	0.02-2.0	2	73.7	N/A
Study code:	BSF 605/002847	Barley	Italy	BAS 351 32 H: 1 x 1.55	37-39	0	shoots	8.66	0.555	0.109	9.28	ears	0.02-2.0	2	100.4	N/A
Doc ID:	2000/1018490					20	ears	0.020	0.116	<0.02	0.15	straw	0.02-2.0	2	100.8	N/A
Trial No.	BSF/605-4					20	remaining shoots	<0.02	1.40	0.020	1.35	shoots	0.02-2.0	2	83.7	N/A
GLP:	yes					65	grains	<0.02	<0.02	<0.02	<0.06	grain	0.02-0.2	2	89.3	N/A
Year	1999					65	straw	0.042	0.046	<0.02	*0.10*					

Table 6.3/123 Residues of bentazone in barley after one application of BAS 351 32 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	BSF 605/002847	Barley	Italy	BAS 351 32 H: 1 x 1.55	39	0	shoots	4.82	0.278	0.027	5.11					
Doc ID:	2000/1018490					17	ears	0.029	0.192	<0.02	0.23					
Trial No.	BSF/605-5					17	remaining shoots	<0.02	0.614	<0.02	0.61					
GLP:	yes					57	grains	<0.02	<0.02	<0.02	<u>≤0.06</u>					
Year	1999					57	straw	0.062	0.061	<0.02	*0.14*					

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938; for calculation purposes, residues were treated as follows: <0.02 + <0.02 + <0.02 = <0.06
6) at application
_ underlined values were used for MRL calculation
N/A not applicable
values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Wheat

Report:	II A 6.3.8/10 Oxspring S. 2011(c) Study on the behaviour of Bentazone in wheat after treatment with BAS 351 32 H in Northern Europe during 2010 BASF DocID 2011/1059497
Guidelines:	None
GLP:	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

During the 2010 growing season, eight field trials were conducted in representative wheat growing areas in Northern Europe to determine the residue level of bentazone, 6-OH- and 8-OH-bentazone in or on raw agricultural commodities (RAC). Plot 2 was treated with BAS 351 32 H, an SL formulation of bentazone, and was foliar applied once at a rate corresponding to 0.96 kg a.s./ha at BBCH 32. Whole plant specimens were collected immediately after application (0 DALA) and at BBCH 49. Ears and rest of plant without roots were then taken 59-67 DALA (crop growth stage BBCH 71-83) and finally grain and straw specimens were taken at normal commercial harvest (BBCH 89).
Specimens were analysed for bentazone, 6-OH- and 8-OH-bentazone using BASF Method No. 438/2 (L0044/02). The limit of quantitation was 0.01 mg/kg for all analysed substances. The study showed that no quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable in wheat grains and therefore the total bentazone residues did not exceed 0.03 mg/kg in any investigated sample.

I. MATERIAL AND METHODS

A. MATERIALS

1. **Test Material:**
   Description: BAS 351 32 H (SL)
   Lot/Batch #: 70896356PO
   Purity: 480 g/L BAS 351 H, nominal
   CAS#: 25057-89-0
   Development code:
   Spiking levels: 0.01-80 mg/kg (Bentazone, 6-OH-bentazone, 8-OH-bentazone)

2. **Test Commodity:**
   Crop: Wheat
   Type: Cereals
   Variety: Akteur, Robigus, Courtot, Tabasco, Oakley, Tobasco, Frument, Suba
   Botanical name: *Triticum aestivum*
   Crop parts(s) or processed commodity: Whole plant, ears, rest of plant without roots, grain, straw
   Sample size: ≥0.5-1 kg

B. STUDY DESIGN

1. Test procedure

During the 2010 growing season, eight field trials were conducted in representative wheat growing areas in the UK, Germany, France, The Netherlands and Denmark to determine the residue level of bentazone, 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
The trials consisted of an untreated and a treated plot. Wheat was treated once with BAS 351 32 H, an SL formulation of bentazone (480 g a.s./L), as a foliar application at a rate corresponding to 0.96 kg a.s./ha at BBCH 32. The nominal spray volume used was 200 L/ha. Whole plant specimens were collected immediately after application (0 DALA) at crop growth stages 32 and 49. Ears and rest of plant without root samples were then taken 59-67 DALA (crop growth stage BBCH 71-83) and finally grain and straw specimens were taken at normal commercial harvest (BBCH 89) at DALA 87-103. The maximum storage interval from harvest to extraction was 350 days.

Table 6.3/124: Target application rates and timings for wheat

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2010	8	1	F	BAS 351 32 H (SL)	BAS 351 H	0.96	200	BBCH 32

2. Description of analytical procedures

Specimens were analysed for bentazone, 6-OH- and 8-OH-bentazone using BASF Method No. 438/2 (L0044/02). Bentazone and its metabolites are extracted from plant matrices with aqueous methanol. After purification by an iso-octane liquid-liquid partition, hydrolysis using enzymatic cleavage is performed. After a $Ca(OH)_2$-precipitation step to remove acidic plant constituents, a reversed phase C_{18}-SPE clean-up is performed. The final determination of the residues of bentazone and its hydroxy metabolites is performed by LC-MS/MS. The limit of quantitation was 0.01 mg/kg for all analysed substances.

II. RESULTS AND DISCUSSION

The residue ranges of bentazone, 6-OH-bentazone and 8-OH-bentazone in wheat commodities treated once with the formulation BAS 351 32 H are shown in Table 6.3/125. Details can be found in Table 6.3/126.
The bentazone residues in wheat whole plant specimens taken directly after application ranged between 24 and 60 mg/kg. At BBCH 49-59 the residues in whole plant specimens had declined to a range between <0.01 and 0.04 mg/kg. At about 60 DALA no residues of bentazone were detectable in the ear specimens, whereas in rest of plant without roots specimens residues between <0.01 and 0.01 mg/kg were found. At harvest (BBCH 89), no residues above the LOQ were found in the grain and straw specimens.
The 6-OH-bentazone residues in wheat whole plant specimens taken directly after application ranged between 0.53 and 2.0 mg/kg. At BBCH 49-59 the residues in whole plant specimens ranged between 0.08 and 1.4 mg/kg. At about 60 DALA no residues of 6-OH-bentazone were determined in ears, and between 0.03 and 0.12 mg/kg were found in the rest of plant without roots specimens. At harvest (BBCH 89) no residues were detected in the grain specimens, but residues of between <0.01 and 0.04 mg/kg were found in the straw specimens.
The 8-OH-bentazone residues in wheat whole plant specimens taken directly after application ranged between 0.07 and 0.39 mg/kg. At BBCH 49-59 no detectable residues were found in the whole plant specimens. At about 60 DALA no residues of 8-OH-bentazone were detected in the ear and the rest of plant without roots specimens. At harvest (BBCH 89) no residues were detected in the grain and straw specimens.
The total bentazone residues in wheat whole plant specimens taken directly after application ranged between 25 and 62 mg/kg. At BBCH 49-59 the residues in whole plant specimens ranged between 0.09 and 1.3 mg/kg. At about 60 DALA no residues above the limit of detection were found in the ear specimens, whereas in the rest of plant without roots specimens, residues between 0.05 and 0.13 mg/kg were found. At harvest (BBCH 89) no residues were detected in the grain specimens, whereas in the straw specimens, residues between <0.03 and 0.06 mg/kg were found.
No residues of bentazone or its metabolites 6-OH- and 8-OH-bentazone above the LOQ were found in the control specimens.

Table 6.3/125: Summary of residues in wheat

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	2010	0	32	whole plant	24-60	0.53-2.0	0.07-0.39	25-62
		22-36	49-59	whole plant	<0.01-0.04	0.08-1.4	<0.01	0.09-1.3
		59-67	71-83	ears	<0.01	<0.01	<0.01	<0.03
		59-67	71-83	rest of plant*	<0.01-0.01	0.03-0.12	<0.01	0.05-0.13
		87-103	**89**	**grain**	**<0.01**	**<0.01**	**<0.01**	**<0.03**
		87-103	89	straw	<0.01	<0.01-0.04	<0.01	<0.03-0.06

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
* without root
Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

The study showed that no quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable in wheat grains and therefore the total bentazone residues were below 0.03 mg/kg in all investigated samples.

Table 6.3/126 Residues of bentazone in wheat after one application of BAS 351 32 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	385355	Wheat	Germany	BAS 351 32 H: 1 x 0.96	32	0	whole plant	25	1.0	0.07	26	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2011/1059497					34	whole plant	<0.01	0.08	<0.01	0.09	Bentazone				
Trial No.	L100111					59	ears	<0.01	<0.01	<0.01	<0.03	whole plant	0.01-80	5	97	6.9
GLP:	yes					59	rest plant[5]	<0.01	0.07	<0.01	0.09	ears	0.01-0.10	4	104	4.1
Year	2010					87	grain	<0.01	<0.01	<0.01	<0.03	rest plant[5]	0.01-0.10	4	104	5.9
						87	straw	<0.01	0.04	<0.01	*0.06*	grain	0.01-0.10	4	104	11
Study code:	385355	Wheat	UK	BAS 351 32 H: 1 x 0.96	32	0	whole plant	28	0.78	0.13	29	straw	0.01-0.10	4	106	4.4
Doc ID:	2011/1059497					35	whole plant	<0.01	0.15	<0.01	0.16	6-OH-bentazone				
Trial No.	L100112					61	ears	<0.01	<0.01	<0.01	<0.03	whole plant	0.01-2.0	5	88	13
GLP:	yes					61	rest plant[5]	<0.01	0.07	<0.01	0.09	ears	0.01-0.10	4	91	16
Year	2010					103	grain	<0.01	<0.01	<0.01	<0.03	rest plant[5]	0.01-0.10	4	81	12
						103	straw	<0.01	<0.01	<0.01	*<0.03*	grain	0.01-0.10	4	76	13
Study code:	385355	Wheat	France	BAS 351 32 H: 1 x 0.96	32	0	whole plant	60	1.8	0.39	62	straw	0.01-0.10	4	85	4.6
Doc ID:	2011/1059497					36	whole plant	0.03	0.15	<0.01	0.18	8-OH-bentazone				
Trial No.	L100113					67	ears	<0.01	<0.01	<0.01	<0.03	whole plant	0.01-2.0	5	90	12
GLP:	yes					67	rest plant[5]	<0.01	0.03	<0.01	0.05	ears	0.01-0.10	4	89	16
Year	2010					95	grain	<0.01	<0.01	<0.01	<0.03	rest plant[5]	0.01-0.10	4	82	11
						95	straw	<0.01	0.01	<0.01	0.03	grain	0.01-0.10	4	82	13
Study code:	385355	Wheat	The Netherlands	BAS 351 32 H: 1 x 0.96	32	0	whole plant	30	0.89	0.16	31	straw	0.01-0.10	4	79	1.2
Doc ID:	2011/1059497					30	whole plant	<0.01	0.34	<0.01	0.34					
Trial No.	L100114					59	ears	<0.01	<0.01	<0.01	<0.03					
GLP:	yes					59	rest plant[5]	<0.01	0.06	<0.01	0.08					
Year	2010					91	grain	<0.01	<0.01	<0.01	<0.03					
						91	straw	<0.01	<0.01	<0.01	*<0.03*					

Table 6.3/126 Residues of bentazone in wheat after one application of BAS 351 32 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	385355	Wheat	UK	BAS 351 32 H: 1 x 0.96	32	0	whole plant	24	1.0	0.18	25					
Doc ID:	2011/1059497					23	whole plant	0.04	1.4	<0.01	1.4					
Trial No.	L100115					60	ears	<0.01	<0.01	<0.01	<0.03					
GLP:	yes					60	rest plant[5]	<0.01	0.12	<0.01	0.13					
Year	2010					87	grain	<0.01	<0.01	<0.01	<0.03					
						87	straw	<0.01	<0.01	<0.01	*<0.03*					
Study code:	385355	Wheat	Germany	BAS 351 32 H: 1 x 0.96	32	0	whole plant	26	2.0	0.28	28					
Doc ID:	2011/1059497					29	whole plant	<0.01	0.19	<0.01	0.20					
Trial No.	L100116					61	ears	<0.01	<0.01	<0.01	<0.03					
GLP:	yes					61	rest plant[5]	<0.01	0.04	<0.01	0.06					
Year	2010					91	grain	<0.01	<0.01	<0.01	<0.03					
						91	straw	<0.01	<0.01	<0.01	*<0.03*					
Study code:	385355	Wheat	Denmark	BAS 351 32 H: 1 x 0.96	32	0	whole plant	27	0.68	0.12	28					
Doc ID:	2011/1059497					22	whole plant	<0.01	1.1	<0.01	1.1					
Trial No.	L100117					60	ears	<0.01	<0.01	<0.01	<0.03					
GLP:	yes					60	rest plant[5]	0.01	0.11	<0.01	0.12					
Year	2010					90	grain	<0.01	<0.01	<0.01	<0.03					
						90	straw	<0.01	<0.01	<0.01	*<0.03*					
Study code:	385355	Wheat	France	BAS 351 32 H: 1 x 0.96	32	0	whole plant	31	0.53	0.13	32					
Doc ID:	2011/1059497					28	whole plant	0.02	1.2	<0.01	1.2					
Trial No.	L100118					60	ears	<0.01	<0.01	<0.01	<0.03					
GLP:	yes					60	rest plant[5]	<0.01	0.06	<0.01	0.08					
Year	2010					87	grain	<0.01	<0.01	<0.01	<0.03					
						87	straw	<0.01	<0.01	<0.01	*<0.03*					

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
   for calculation purposes, residues were treated as follows: <0.01 + <0.01 + <0.01 = <0.03
5) without roots
6) at application
_ underlined values were used for MRL calculation
values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Report:	II A 6.3.8/11 Blaschke U.G. 2000(I) BAS 351 32 H: Determination of the magnitude of the residue of Basagran (BAS 351 32 H) applied to wheat in Southern Europe in 1998 BASF DocID 2000/1018487
Guidelines:	EEC 91/414; FAO Guidelines Rome 1990; IVA Guidelines for Residue Studies Sections IA and IB 2nd edition 1992; EEC 87/18
GLP:	Yes (laboratory certified by Department of Health of the Government of the United Kingdom, United Kingdom)

Executive Summary

A total number of 4 field trials were conducted at different representative wheat growing areas in Southern Europe (Spain and Italy) during 1998. The herbicidal product BAS 351 32 H (485 g/L BAS 351 H) was applied at an application rate of 3.2 L/ha corresponding to an application rate of 1.55 kg a.s./ha. The first samples (shoots, roots) were collected directly after the last application and subsequent samples were taken on day 16-28 (ears, shoots, roots) and 70-78 days (grain, roots, straw). Specimens were analysed by means of BASF Method No. 438/1 (which is a miniaturised version of BASF Method No. 438/0). The limit of quantification was 0.02 mg/kg for all analysed substances. The study showed that no quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable in wheat grains and therefore the total bentazone residues (expressed as bentazone equivalents) were below 0.06 mg/kg in all investigated grain samples. Therefore, the residue values were used for MRL determination (see chapter 6.7) even though an exaggerated application rate compared to the critical GAP was used.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 32 H (SL)
Lot/Batch #:	98-1
Purity:	485 g/L BAS 351 H
CAS#:	25057-89-0
Development code:	
Spiking levels:	0.02-2.0 mg/kg (Bentazone, 6-OH-bentazone, 8-OH-bentazone)

2. Test Commodity:

Crop:	Wheat
Type:	Cereals
Variety:	Vitron, Cajeme, Excel, Centauto
Botanical name:	*Triticum aestivum*
Crop parts(s) or processed commodity:	Shoots, ears, grain, straw
Sample size:	0.5 to 5.5 kg

B. STUDY DESIGN

1. Test procedure

A total number of 4 field trials were conducted at different representative wheat growing areas in Southern Europe (Spain and Italy) during 1998. The herbicidal product BAS 351 32 H (485 g/L BAS 351 H) was applied at an application rate of 3.2 L/ha corresponding to an application rate of 1.55 kg as/ha. The first samples (shoots, roots) were collected directly after the last application and subsequent samples were taken on day 16-28 (ears, shoots, roots) and 70-78 days (grain, roots, straw). Collected samples were stored frozen at -20°C until further analysis.

Table 6.3/127 Target application rates and timings for wheat

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
1998	4	1	F	BAS 351 32 H (SL)	BAS 351 H	1.55	300	BBCH 32-34

2. Description of analytical procedures

Residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were determined following BASF Method No. 438/1 (which is a miniaturised version of BASF Method No. 438/0). Bentazone and its metabolites are extracted from plant matrices with aqueous methanol. After purification by an iso-octane liquid-liquid partition, the two relevant metabolites which are present as glucosides are hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$-precipitation step to remove acidic plant constituents, a reversed phase C_{18}-column clean-up is performed. The analytes are then methylated with diazomethane and their derivatives are purified using a silica gel-column. The final determination of the residues of bentazone and its hydroxy metabolites is performed by GC/MS. The limit of quantitation was 0.02 mg/kg for all analysed substances.

II. RESULTS AND DISCUSSION

The residue ranges of bentazone, 6-OH-bentazone and 8-OH-bentazone in wheat commodities treated once with the formulation BAS 351 32 H are shown in Table 6.3/128. Details can be found in Table 6.3/129.
No quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable in any of the investigated wheat grain samples. As a result, the total residues of bentazone (expressed as bentazone equivalents) in grains were below 0.06 mg/kg in all field trials. Highest residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were measured in wheat shoots taken directly after the last application resulting in total bentazone residues (expressed as bentazone equivalents) ranging from 20.28-47.28 mg/kg and in roots with total bentazone residues (expressed as bentazone equivalents) ranging from 0.06-3.03 mg/kg. The total bentazone residues level (expressed as bentazone equivalents) detected in ears ranged from <0.06-1.49 mg/kg and ranged from 0.08-0.10 mg/kg in straw.

Table 6.3/128 Summary of residues in wheat

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU South	1998	0	not reported	shoots	16.72-42.95	2.92-7.84	0.067-0.182	20.28-47.28
		16-28		shoots w/o ears	0.045-1.34	1.77-4.39	<0.02-0.045	1.76-5.50
		16-28		ears	<0.02-0.332	<0.02-1.21	<0.02	<0.06-1.49
		0		roots	0.065-2.94	0.021-0.047	<0.02-0.067	0.13-3.03
		16-28		roots	<0.02-0.542	<0.02-0.096	<0.02-0.028	0.10-0.66
		70-78		roots	<0.02	<0.02-0.054	<0.02-0.024	<0.06-0.09
		71-78		**grain**	**<0.02**	**<0.02**	**<0.02**	**<0.06**
		71-78		straw	0.031-0.035	0.032-0.057	<0.02	0.08-0.10

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

The study showed that no quantifiable residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detectable in wheat grains and therefore the total bentazone residues (expressed as bentazone equivalents) were below 0.06 mg/kg in all investigated samples.

Table 6.3/129 Residues of bentazone in wheat after one application of BAS 351 32 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	BSF 590/984865	Wheat	Spain	BAS 351 32 H: 1 x 1.55	32-33	0	shoots	33.47	7.84	0.067	40.89	**Method:** No. 438/1; LOQ = 0.02 mg/kg				
Doc ID:	2000/1018487					0	roots	2.85	0.034	0.058	2.94	Bentazone				
Trial No.	BSF/590-1					28	ears	<0.02	0.250	<0.02	0.27	shoots	0.02-2.0	2	98.7	N/A
GLP:	yes					28	shoots*	0.045	3.53	0.023	3.38	ears	0.02-2.0	2	102.3	N/A
Year	1998					28	roots	0.087	0.055	<0.02	0.16	shoots*	0.02-2.0	2	109.1	N/A
						78	grain	<0.02	<0.02	<0.02	<u><0.06</u>	grain	0.02-2.0	3	102.2	16.9
						78	roots	<0.02	0.025	0.022	0.06	straw	0.02-0.2	2	106.4	N/A
						78	straw	0.032	0.038	<0.02	*0.09*	roots	0.02-2.0	3	105.7	6.6
Study code:	BSF 590/984865	Wheat	Spain	BAS 351 32 H: 1 x 1.55	32-33	0	shoots	42.95	4.43	0.182	47.28	6-OH-bentazone				
Doc ID:	2000/1018487					0	roots	2.94	0.028	0.067	3.03	shoots	0.02-2.0	2	70.5	N/A
Trial No.	BSF/590-2					28	ears	<0.02	<0.02	<0.02	<0.06	ears	0.02-2.0	2	84.8	N/A
GLP:	yes					28	shoots*	0.060	3.14	<0.02	3.02	shoots*	0.02-2.0	2	84.7	N/A
Year	1998					28	roots	0.079	<0.02	<0.02	0.12	grain	0.02-2.0	3	95.8	17.7
						78	grain	<0.02	<0.02	<0.02	<u><0.06</u>	straw	0.02-0.2	2	94.2	N/A
						78	roots	<0.02	0.054	0.023	0.09	roots	0.02-2.0	3	89.1	14.7
						78	straw	0.031	0.057	<0.02	*0.10*	8-OH-bentazone				
Study code:	BSF 590/984865	Wheat	Italy	BAS 351 32 H: 1 x 1.55	34	0	shoots	17.46	2.92	0.091	20.28	shoots	0.02-2.0	2	76.0	N/A
Doc ID:	2000/1018487					0	roots	0.153	0.021	<0.02	0.19	ears	0.02-2.0	1	71.7	N/A
Trial No.	BSF/590-3					16	ears	0.332	1.21	<0.02	1.49	shoots*	0.02-2.0	2	98.5	N/A
GLP:	yes					16	shoots*	1.34	4.39	0.045	5.50	grain	0.02-2.0	1	96.3	N/A
Year	1998					16	roots	0.542	0.096	0.028	0.66	straw	0.02-0.2	2	79.0	N/A
						70	roots	<0.02	<0.02	<0.02	<0.06	roots	0.02-2.0	3	85.0	9.6

Table 6.3/129 Residues of bentazone in wheat after one application of BAS 351 32 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	BSF 590/984865	Wheat	Italy	BAS 351 32 H: 1 x 1.55	32	0	shoots	16.72	4.07	0.105	20.64					
Doc ID:	2000/1018487					0	roots	0.065	0.047	0.024	0.13					
Trial No.	BSF/590-4					19	ears	<0.02	<0.02	<0.02	<0.06					
GLP:	yes					19	shoots*	0.070	1.77	0.033	1.76					
Year	1998					19	roots	<0.02	0.055	0.025	0.10					
						71	grain	<0.02	<0.02	<0.02	<u>≤0.06</u>					
						71	roots	<0.02	<0.02	0.024	0.06					
						71	straw	0.035	0.032	<0.02	*0.08*					

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938; for calculation purposes, residues were treated as follows: <0.02 + <0.02 + <0.02 = <0.06
6) at application
_ underlined values were used for MRL calculation
N/A not applicable
* without ears
values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

6.3.9 Herbal infusions (dried)

Valerian root

Table 6.3/130 GAP for the use of BAS 351 H in/on valerian root

Crop	Maximum Applied Dose	Water Volume	PHI	Application Method	Application Timing
Valerian (root)	1 x 0.960 kg BAS 351 H/ha	200-400L/ha	F	spray application	13-15

PHI = pre-harvest interval

Table 6.3/131 GAP information of residue trials conducted in valerian roots during 2001-2003

Crop	Region	Country	Formulation	Application[0]				DALA[1]
				Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
Valerian roots	EU North	Germany (4 trials)	Basagran (BAS 351 32 H): 480 g/L Bentazone	spray application	0.960	0.32	1	134-162

0) actual application rates varied by 10% at most
1) days after last application

Report: II A 6.3.9/1
Kludssuweit H. 2004(b)
Untersuchung auf Rueckstaende von Bentazon in Baldrian
BASF DocID 2004/1037134
Guidelines: None
GLP: Yes
(laboratory certified by Ministerium fuer Landwirtschaft und Umwelt des Landes Sachsen-Anhalt, Magdeburg, Germany)

Note: An English translation of the report is provided in DocID 2011/1072552

Executive Summary

During the growing seasons of 2001-2003, 4 field trials were conducted with valerian root in Germany, to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
Therefore, the formulated product Basagran (48% bentazone, SL) was applied once at a rate equivalent to 0.960 kg a.s./ha of bentazone in a spray volume of 300 L/ha. Each trial consisted of a control (untreated) and a treated plot. The application was done at growth stages BBCH 12-23. Specimens of valerian root were collected 134, 154, 155 and 162 days after the last application (DALA) at growth stages BBCH 31-47.
The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone using a modified analytical method GLP-AG 2, based on the BASF Method No. 197 (1982), which allows the determination of the analytes by GC-PFPD (Gas Chromatograph-Pulsed Flame Photometric Detector) with a limit of quantitation (LOQ) of 0.048 and 0.045 mg/kg for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone (expressed as parent equivalent), respectively.
No residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone above the limit of quantitation (<0.048, <0.045 mg/kg) were found in all valerian root specimens investigated.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 32 H, Basagran (SL)
Lot/Batch #:	Not reported, 480 g/L bentazone, nominal
Purity:	
CAS#:	25057-89-0 (bentazone)
Development code:	
Spiking levels:	0.045-0.225 mg/kg

2. Test Commodity:

Crop:	Valerian (roots)
Type:	Herbal infusions (dried)
Variety:	MB 001, 54
Botanical name:	*Valeriana officinalis*
Crop parts(s) or processed commodity:	- Roots dried
Sample size:	1.0 kg

B. STUDY DESIGN

1. Test procedure

During the 2001-2003 growing season, 4 field trials were conducted with valerian root in Germany to determine the residue level of bentazone (BAS 351 H) and its metabolites BH 351-6-OH and BH 351-8-OH in or on raw agricultural commodities (RAC).
Therefore, the formulated product Basagran (480 g/L bentazone, SL) was applied once at a rate equivalent to 0.960 kg a.s./ha of bentazone in a spray volume of 300 L/ha. Each trial consisted of a control (untreated) and a treated plot. The application was done at growth stages BBCH 12-23. Specimens of valerian root were collected 134, 154, 155 and 162 days after the last application (DALA) at growth stages BBCH 31-47.

Table 6.3/132 Target application and timings for valerian root

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2001-2003	4	1	F	BAS 351 32 H (SL)	BAS 351 H	0.960	300	BBCH 12-23

2. Description of analytical procedures

The specimens were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone using a modified analytical method GLP-AG 2, based on the BASF Method No. 197 (1982), which allows the determination of the analytes by GC-PFPD (Gas Chromatograph-Pulsed Flame Photometric Detector) with a limit of quantitation (LOQ) of 0.048 and 0.045 mg/kg for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone, respectively.
Residues were extracted from valerian root specimens with methanol, followed by hydrolysis with methanolic hydrochloric acid. After a liquid/liquid partition against dichloromethane and methylation with iodomethane, the samples were cleaned up on a florisil mini-column. The final determination was performed by GC-PFPD (Gas Chromatograph-Pulsed Flame Photometric Detector).

II. RESULTS AND DISCUSSION

The summary of residues in valerian roots is shown in Table 6.3/133, detailed residue levels are shown in Table 6.3/134.
No residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone above the limit of quantitation (<0.048 mg/kg, <0.045 mg/kg) were found in all valerian root specimens investigated.

Table 6.3/133 Summary of residues in valerian root

Region	Year	DALA[1]	Growth Stage (BBCH)[2]	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Benta-zone[3]	8-OH-Benta-zone[4]	Total Benta-zone[5]
EU North	2001-2003	**134**	**47**	**valerian roots (dried)**	**<0.048**	**<0.045**	**<0.045**	**<0.13**
		162	**31**		**<0.048**	**<0.045**	**<0.045**	**<0.13**
		155	**47**		**<0.048**	**<0.045**	**<0.045**	**<0.13**
		154	**39**		**<0.048**	**<0.045**	**<0.045**	**<0.13**

1) days after last application
2) growth stage at sampling
3) expressed as parent equivalent
4) expressed as parent equivalent
5) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

No residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone above the limit of quantitation (LOQ) were found in all valerian root specimens investigated.

Table 6.3/134 Residues of bentazone in valerian roots after one application of Basagran (48% bentazon, SL) in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Benta-zone	6-OH-Benta-zone[3]	8-OH-Benta-zone[4]	Total Benta-zone[5]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	LPSA-BENT-BALD-03	Valerian roots	Germany	Basagran: 1 x 0.960	23	134	dried roots	<0.048	<0.045	<0.045	<0.13	**Method:** GLP-AG 2				
Doc ID:	2004/1037134											LOQ = 0.048, 0.045, 0.045 mg/kg (bentazone, 6-OH-, 8-OH-bentazone)				
Trial No.	HBa0101/1/2											bentazone				
GLP:	yes											roots	0.048-0.240	8	104	6
Year	2001											6-OH-bentazone				
Study code:	LPSA-BENT-BALD-03	Valerian roots	Germany	Basagran: 1 x 0.960	17	162	dried roots	<0.048	<0.045	<0.045	<0.13	roots	0.045-0.225	8	91	6
Doc ID:	2004/1037134											8-OH-bentazone				
Trial No.	HBa0102/2/4											roots	0.045-0.225	8	82	7
GLP:	yes															
Year	2002															
Study code:	LPSA-BENT-BALD-03	Valerian roots	Germany	Basagran: 1 x 0.960	12	155	dried roots	<0.048	<0.045	<0.045	<0.13					
Doc ID:	2004/1037134															
Trial No.	HBa0103/2/5															
GLP:	yes															
Year	2003															
Study code:	LPSA-BENT-BALD-03	Valerian roots	Germany	Basagran: 1 x 0.960	13-15	154	dried roots	<0.048	<0.045	<0.045	<0.13					
Doc ID:	2004/1037134															
Trial No.	HBa0103/1/5															
GLP:	yes															
Year	2003															

0) actual application rates varied by 10% at most
1) days after last application
2) growth stage at last application
3) expressed as parent equivalent
4) expressed as parent equivalent
5) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
_ underlined values were used for MRL calculation

6.4 Livestock feeding studies

According to the Appendix G (Lundehn document 7031/VI/95 rev.4, July 1996) and OECD guidelines as well as the EFSA feeding tables a feeding study in livestock is only required,

(1) if significant residues (≥ 0.1 mg/kg of the total diet as received, except special cases, such as active substances which accumulate) occur in crops or part of the crops fed to livestock,

and

(2) if metabolism studies indicate that significant residues (above the limit of determination) may occur in any edible animal tissue, taking into account the residue levels in potential feeding stuff obtained at the 1x dose rate.

Among the intended uses for bentazone in garlic, onions, shallots, beans (legumes and pulses), peas (legumes and pulses), flax, poppy seed, soybean, cereals (wheat, triticale, rye, barley, oats, spelt, sorghum, rice and maize) as well as green forage (e.g. maize or alfalfa/clover), the guideline required uses were used to calculate the maximum intake of bentazone residues.

Since the relevant residue for monitoring is total bentazone (sum of bentazone, 6-OH-bentazone and 8-OH-bentazone) the bentazone equivalents were considered for calculation of poultry dietary feed burden. Use of total bentazone residue values generally overestimates the dietary feed burden. For cattle, in contrast, calculations were done with each of the relevant residues (bentazone, 6-OH- and 8-OH-bentazone, respectively) to get a more realistic picture of the actual dietary feed burden.

The following tables (Table 6.4/1 and Table 6.4/2) are showing the anticipated dietary burden for poultry (chicken) as well as for ruminants (dairy cattle and beef cattle). The values were calculated according to Guideline: "Working document 7031/VI/95 rev. 4 (22 July 1996) Appendix G - Livestock Feeding studies" and related to EFSA feeding tables provided in context with Profile.

Poultry

Table 6.4/1: Estimated maximum dietary burden of total bentazone residues for poultry

Crop	Dry Matter Content (%)	Residue Level (HR)	Poultry*	
			% of diet	mg/kg bw/d
I Green forage				
Maize forage	20	1.78	0	-
II Grains				
Wheat grain	86	0.06#	70	0.003084
V Root + Tuber				
Potatoes	15	0.06	20	0.006737
VI Oil seed				
Rape seed meal	86	0.078##	10	0.000573
Dietary burden:		mg/kg bw/day	0.010	
		mg/animal/day	0.020	
		mg/kg total feed (DM)	0.165	

* feed intake 0.12 kg DM, body weight (bw) 1.9 kg
\# STMR
\## highest STMR of oilseeds (linseed = 0.06 mg/kg) x processing factor (1.3)

Ruminants/ Cattle

Table 6.4/2: Estimated maximum dietary burden of bentazone residues for cattle

Crop	Dry Matter Content (%)	Residue Level (HR, mg/kg)	Dairy Cattle*		Beef Cattle**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Maize forage	20	0.13	100	0.023632	100	0.027852
Dietary burden:	mg/kg bw/day		0.024		0.028	
	mg/animal/day		13.0		9.7	
	mg/kg total feed (DM)		0.65		0.65	

* feed intake 20 kg DM, body weight (bw) 550 kg
** feed intake 15 kg DM, body weight (bw) 350 kg

Table 6.4/3: Estimated maximum dietary burden of 6-OH-bentazone residues for cattle

Crop	Dry Matter Content (%)	Residue Level (HR, mg/kg)	Dairy Cattle*		Beef Cattle**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Maize forage	20	1.79	100	0.325515	100	0.383570
Dietary burden:	mg/kg bw/day		0.33		0.38	
	mg/animal/day		179.0		134.2	
	mg/kg total feed (DM)		8.95		8.95	

* feed intake 20 kg DM, body weight (bw) 550 kg
** feed intake 15 kg DM, body weight (bw) 350 kg

Table 6.4/4: Estimated maximum dietary burden of 8-OH-bentazone residues for cattle

Crop	Dry Matter Content (%)	Residue Level (HR, mg/kg)	Dairy Cattle*		Beef Cattle**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Maize forage	20	0.08	100	0.01454545	100	0.0171429
Dietary burden:	mg/kg bw/day		0.015		0.017	
	mg/animal/day		8.0		6.0	
	mg/kg total feed (DM)		0.40		0.40	

* feed intake 20 kg DM, body weight (bw) 550 kg
** feed intake 15 kg DM, body weight (bw) 350 kg

6.4.1 Poultry

As the calculation in Table 6.4/1 shows, the anticipated dietary burden levels are 0.020 mg/animal/day for chicken.

The current guideline requires anticipating the residues with dosing at expected dietary burden. As a worst case, residues from the metabolism studies in chicken were compared to the 1x level of chicken.

The metabolism study in hens was performed at an actual dose level of 10 mg/animal/day. The respective estimated dietary burden for chicken is 0.020 mg/animal/day. This results in an overdosing factor of 500. With this factor, the residues were anticipated by extrapolation of the total radioactive residues observed in the metabolism study. For poultry, total bentazone (sum of bentazone, 6-OH-bentazone and 8-OH-bentazone) residue levels were considered. Use of total bentazone residue values generally overestimates the dietary feed burden.

Table 6.4/5: Extrapolated residues of total bentazone in poultry tissues and eggs

Poultry Commodity	Residues (Metabolism Study)* [mg/kg bentazone equivalents]	Anticipated Residues with a Dosing at the 1x Dietary Burden [mg/kg bentazone equivalents]
Subcutaneous fat	0.15	0.0003
Peritoneal fat	0.06	0.0001
Leg Muscle	0.48	0.0010
Breast muscle	0.41	0.0008
Liver	1.46	0.0029
Eggs (max.)	0.20	0.0004

* bentazone equivalents = bentazone + 6-OH-bentazone + 8-OH-bentazone

As Table 6.4/5 shows, dosing at an equivalent of 1x of the estimated dietary burden for poultry would result in residues far below the LOQ of the method (0.06 mg/kg) for all commodities required according to the current guideline.
In such cases, the metabolism study can serve as a feeding study and thus no separate magnitude of residues study in poultry is required.

6.4.2 Lactating ruminants (goat or cow)

The calculation resulted in an anticipated 1x dietary burden of 0.38 mg/kg and 0.33 mg/kg bw per day (based on 6-OH-bentazone which showed highest residue levels) for beef and dairy cattle, respectively. Again, residues with dosing at the equivalent of the dietary burden were extrapolated from the three metabolism studies in ruminants (one each for bentazone, 6-OH- and 8-OH-bentazone). As a worst case, residue calculation was based on the 1x level for beef cattle, which covers the lower 1x level for dairy cattle as well. All three metabolism studies included a highly overdosed goat which is not considered in the calculations.

One goat metabolism study was performed with bentazone nominal at a dose level of 3 mg/kg bw per day (corresponding to 123 mg/kg feed; for details please refer to document M, chapter 4.2.1 of the Annex II dossier 1995). The respective estimated dietary burden of bentazone for beef cattle is 0.028 mg/kg bw per day. This results in an overdosing factor of 107.14. With this factor, the residues were anticipated by extrapolation of the total radioactive residues observed in the metabolism study:

Table 6.4/6: Extrapolated residues of bentazone in lactating ruminants tissues and milk (overdosing 107.14)

Edible Commodity	Residues (Metabolism Study) [mg/kg]	Anticipated Residues with a Dosing at the 1x Dietary Burden [mg/kg]
Milk	0.027	0.0003
Liver	0.03	0.0003
Kidney	0.6	0.005
Muscle	0.013	0.0001
Fat	1.57	0.015

One goat metabolism study was performed with 6-OH-bentazone nominal at a dose level of 2 mg/kg bw per day (corresponding to 41 mg/kg feed; for details please refer to document M, chapter 4.2.1 of the Annex II dossier 1995). The respective estimated dietary burden of 6-OH-bentazone for beef cattle is 0.384 mg/kg bw per day. This results in an overdosing factor of 5.21. With this factor, the residues were anticipated by extrapolation of the total radioactive residues observed in the metabolism study:

Table 6.4/7: Extrapolated residues of 6-OH-bentazone in lactating ruminants tissues and milk (overdosing 5.21)

Edible Commodity	Residues (Metabolism Study) [mg/kg]	Anticipated Residues with a Dosing at the 1x Dietary Burden [mg/kg]
Milk	0.021	0.004
Liver	0.018	0.003
Kidney	0.140	0.027
Muscle	0.011	0.002
Fat	0.027	0.005

One goat metabolism study was performed with 8-OH-bentazone nominal at a dose level of 2 mg/kg bw per day (corresponding to 42 mg/kg feed; for details please refer to document M, chapter 4.2.1 of the Annex II dossier 1995). The respective estimated dietary burden of 8-OH-bentazone for beef cattle is 0.017 mg/kg bw per day. This results in an overdosing factor of 117.65. With this factor, the residues were anticipated by extrapolation of the total radioactive residues observed in the metabolism study:

Table 6.4/8: Extrapolated residues of 8-OH-bentazone in lactating ruminants tissues and milk (overdosing 117.65)

Edible Commodity	Residues (Metabolism Study) [mg/kg]	Anticipated Residues with a Dosing at the 1x Dietary Burden [mg/kg]
Milk	0.023	0.0002
Liver	0.021	0.0002
Kidney	0.118	0.001
Muscle	0.012	0.0001
Fat	0.007	< 0.0001

The anticipated residues of bentazone and the relevant metabolites (Tables 6.4/4-6) were added up, to calculate anticipated residues of total bentazone (sum of bentazone and the conjugates of 6-OH-bentazone and 8-OH-bentazone expressed as bentazone):

Table 6.4/9: Sum of extrapolated residues corresponding to total bentazone (sum of bentazone and the conjugates of 6-OH-bentazone and 8-OH-bentazone expressed as bentazone) residues in ruminants tissues and milk

Edible Commodity	Anticipated Residues of total Bentazone in Ruminants [mg/kg]
Milk	0.004
Liver	0.003
Kidney	0.03
Muscle	0.002
Fat	0.02

As Table 6.4/8 shows, dosing at an equivalent of the estimated dietary burden for ruminants would result in residues below or at the LOQ of the enforcement method (0.03 mg/kg) for all edible commodities. The high value for kidney is most likely caused by incomplete excretion at the time of sacrifice which is not expected under practical conditions. Furthermore anticipated dietary burden calculations for cattle include worst-case assumptions such as feeding with the highest residue (HR) for each single residue (bentazone and the two OH-metabolites, respectively) observed in different residue trials. Also, it is unlikely that all feed is treated with bentazone, which is the basic assumption for feed intake calculations.
For the abovementioned calculations and arguments no quantifiable residues would be anticipated in milk and other cattle products as a result of the proposed use.
In such cases, the metabolism study can serve as a feeding study and thus no separate cow feeding study is required. Even if no residues are expected in products of animal origin a goat feeding study was conducted for bentazone and 6-OH-bentazone and considered appropriate in the initial evaluation. This study confirmed that no residues are expected in milk.

6.4.3 Pigs

A feeding study in pigs is required only when the metabolic pathways differ significantly in pigs as compared to ruminants. Because the metabolite patterns in rodents (rats) and ruminants (goats) did not differ significantly, no significant differences in metabolic pathways are expected in pigs.

6.4.4 Fish

Not relevant

6.5 Effects of industrial processing and/or household preparation on

6.5.1 The nature of residue

The hydrolysis study under different processing conditions of ^{14}C-bentazone was no guideline requirement in 1995 when the original Annex II Dossier (1995) was submitted. Since residues in matrices relevant for processing (e.g. soybean seeds, cereal grain; see also M-II, 6.3) are generally very low, a hydrolysis study simulating different processing conditions is not deemed necessary.
Furthermore, no available hydrolysis data indicates any degradation of bentazone, e.g. at pH 5-9 and 25°C, bentazone has been proven to be robust.

6.5.2 Distribution of the residue in peel/pulp

This is not applicable for the bentazone uses.

6.5.3 Residue levels - balance studies on set of representative processes

Processing studies are required when the residues found in the RAC are greater than 0.1 mg/kg or the TMDI is greater 10%. However, even though these criteria are not met by bentazone, processing studies in soybean, maize and rice were included in the Annex II Dossier of 1995 and evaluated in the Full Report (1998). No accumulation was observed for any commodity intended for human consumption.
For the re-registration process no new studies were conducted.

6.5.4 Residue levels - follow-up studies: concentration or dilution factors

No supplementary study on the effects of processing (magnitude of the residue) was performed.

* Full Report of the Peer Review Meetings organised by the ECCO teams for Bentazone (1998)

6.6 Residues in succeeding crops

Data on residues in succeeding crops were reviewed at EU level during the Annex I inclusion process and were considered to be acceptable.
The following EU conclusion was reached in the course of the Annex I inclusion based on a ^{14}C-study performed in chard, turnip, sorghum, radish and wheat as representative crops. The relevant endpoint was copied from the Full Report (Full Report of the Peer Review Meetings organised by the ECCO teams for Bentazone (1998)).

Metabolism in plants (Annex IIA, point 6.1 and 6.7, Annex IIIA, point 8.1 and 8.6)

Rotational crops	Chard, turnip, sorghum, radish, wheat

Residues in succeeding crops (Annex IIA, point 6.6, Annex IIIA, point 8.5)

0.05* mg/kg

Nevertheless, new analytical techniques and implementation of new guidelines for rotational crop studies made higher ID rates and use of state-of-the-art analytics for identification of metabolites desirable. Therefore it was agreed with former RMS Germany to conduct an additional confined rotational crop study to verify the results of the previous study. This new rotational crop study is summarised under 6.6.2 below.

An overview of all metabolites including structures, names, codes and synonyms can be found in Tab 6.11/1 (M-II, 6.11).

6.6.1 Theoretical consideration of the nature and level of the residue

Not relevant.

6.6.2 Metabolism and distribution studies on representative crops

Report:	II A 6.6.2/1 Radzom M. et al. 2011(a) Confined rotational crop study with BAS 351 H BASF DocID 2010/1143715
Guidelines:	EPA 860.1850: Confined Accumulation in Rotational Crops; EPA 860.1000: EPA Residue Chemistry Test Guidelines; PMRA Residue Chemistry Guidelines Section 97.13 Confined Accumulation in Rotational Crops (Canada); BBA IV 3-10; EEC 7524/VI/95 rev. 2 (July 22 1997); OECD 502 Metabolism in Rotational Crops (January 2007)
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

A confined rotational crop study was conducted with U-^{14}C-phenyl-labeled BAS 351 H (bentazone). The active substance was applied to bare silty loamy sand soil in plastic containers at an application rate of 1 x 1000 g a.s./ha using an automatic spray track system. The nature and the level of radioactive residues were investigated in lettuce (immature and mature), white radish (top and root) and spring wheat (forage, hay, straw and grain) after plant back intervals of 30, 120 and 365 days. Plant samples were harvested at maturity, and additional immature lettuce samples as well as spring wheat forage samples (in part dried to hay) were taken 27 to 40 days and 56 to 74 days after planting or sowing, respectively. Soil samples were taken after ploughing and after harvest of the mature crops for each plant back interval. The sampled material was stored in a freezer. All plant samples were homogenised and the radioactive residues in these samples and in the soil samples were determined by combustion analysis.

Significant translocation of radioactive residues from soil into the plants was observed for the plant back interval of 30 DAT which declined rapidly for longer aging periods of 120 and 365 days. The residue concentration in the top soil layer after aging and ploughing slightly decreased with increasing plant back intervals.
The total radioactive residues (TRR) in lettuce (immature and mature samples) did not exceed 0.128 mg/kg for all plant back intervals. The TRR in white radish top was 0.168 mg/kg at a plant back interval of 30 DAT, decreasing to 0.019 mg/kg after 120 DAT and to 0.003 mg/kg (TRR combusted) after 365 DAT. The total radioactive residues in roots of mature crop decreased from 0.128 mg/kg (30 DAT), to 0.012 mg/kg (120 DAT) and finally to 0.001 mg/kg (365 DAT, TRR combusted). In spring wheat, the highest residue levels were measured in hay (ranging from 0.070 to 1.591 mg/kg, for 30 DAT and 365 DAT, respectively) and straw (0.049 to 1.107 mg/kg, for 30 DAT and 365 DAT, respectively). Total radioactive residues in grain accounted for 0.041 to 0.711 mg/kg.

The extractability of the radioactive residues with methanol and water ranged from 43.7% to 71.3% TRR for lettuce and white radish. For spring wheat matrices, the extractability was relatively low with 8.5% to 30.0%, indicating oncorporation of radioactivity in plant constituents. The major portions of the radioactive residues were generally extracted with methanol, except for spring wheat grain where similar portions were extracted with methanol and water.

In all the crop matrices analysed, considerable amounts of the radioactive residues were not extractable with methanol and water. The residual radioactive residues after solvent extraction of all matrices of the plant back intervals 30 DAT and 120 DAT were further characterised using a sequential solubilisation procedure including solubilisation with aqueous ammonia, macerozyme / cellulase and glucosidase / hesperidinase. These incubations were, where applicable, followed by treatments with amylase / amyloglucosidase, tyrosinase / laccase, microwave incubation, and treatments with hydrochloric acid and sodium hydroxide. The solubilised residues had possibly been associated with or embedded / incorporated in insoluble plant material (e.g. proteins, cell wall polymers and starch).
Analysis of the extracts and solubilisates after solvent extraction using the HPLC resulted for all crops, matrices and DAT intervals in an early eluting peak or peak group. This peak or peak group represented the main component in all matrices and for all aging intervals and was identified as polar fraction. The composition of the polar fraction was further investigated using an HPLC method suitable for saccharide analysis. The main portion of the residues of corresponded to glucose, fructose and sucrose (>50% ROI), showing composition of the polar fraction of carbohydrates.

Bentazone and/or its soil metabolites were taken up by and transformed in the rotational crops primarily into sugars (glucose, fructose and sucrose and further components of similar polarity) which were without exception the most abundant components in all matrices examined (methanol / water extracts and solubilisates after solvent extraction). The unchanged parent molecule BAS 351 H was found as minor component in samples of immature (30 DAT) and mature lettuce (30 and 120 DAT) in concentrations of <0.0013 mg/kg and 1.2% TRR only. Additional medium polar degradation products were detected in minor concentrations.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material: ^{14}C BAS 351 H
Description: [Phenyl-U-^{14}C] labelled; specific activity: 5.29 MBq/mg
Lot/Batch #: 210-2201
Purity: 95.2% (HPLC)

CAS#: 25057-89-0
Development code:

Stability of test compound: The test item was stable over the test period

2. Test Commodity:

Crop:	Lettuce	Radish	Wheat
Type:	Not relevant	white	spring
Variety:	Matilda, Giesela	April Cross	Thasos
Botanical name:	*Lactuca Sativa*	*Raphanus sativus*	*Triticum aestivum*
Crop part / processed commodity:	Lettuce head	mature tops mature roots	forage straw hay grain
Sample size:	Not relevant		

3. Soil: A silty loamy sand soil was used. The soil physicochemical properties are described below (see Table 6.6/1).

Table 6.6/1 Soil physicochemical properties

Soil Type	pH	TOC%	Sand%	Silt%	Clay%	Maximal Water Holding Capacity	CEC cmol/kg
Loamy sand	7.5*	1.86	45.6***	38.4***	16.1***	31.7 g/100 g dry weight	16.3

* ($CaCl_2$) *** USDA scheme
TOC total organic carbon
CEC cation exchange capacity

B. STUDY DESIGN

The study was conducted during the period of December 2008 to December 2010 by BASF Agricultural Center, Limburgerhof, Germany.

1. Test procedure

The metabolism of ^{14}C-BAS 351 H (bentazone) in confined rotational crops was investigated after spray application of the test item to bare silty loamy sand soil at a rate of 1 x 1000 g a.s./ha. After application, the soil was aged for 30 days (simulating an emergency plant back; 30 DAT), 120 days (simulating a fall plant back; 120 DAT) and 365 days (365 DAT). Afterwards, the crops lettuce, white radish and spring wheat were sowed or planted.

2. Sampling

Mature and immature lettuce leaves were sampled and the roots remained in the soil. Ripe white radishes were pulled from the soil and separated into the edible parts (root) and the remaining green parts (top). Immature green plants of spring wheat were sampled (wheat forage) and partly dried to wheat hay. In addition, at harvest, mature wheat ears and straw were cut off with scissors. Straw was cut into pieces, and the ears were separated into grain and chaff using a thresher.
Soil samples were taken after the individual plant back intervals and after harvest of the mature crops. All samples were stored in a freezer at -18 °C or below immediately after they were taken and until they were transferred to the metabolism laboratory. The storage conditions stayed the same until analysis started and during the whole period of the study. The extracts were stored in a refrigerator or, for longer periods, in a freezer.

3. Description of analytical procedures

After homogenisation, the total radioactive residues (TRR) of each sample were determined by combustion analysis. All samples were first extracted three times with methanol followed by an additional water extraction (two times). The extracts were combined and measured by liquid scintillation counting (LSC). The combined results of methanol extractions and water extractions are referred to as extractable radioactive residues (ERR). Subsamples of the methanol extracts of plant matrices after a plant back interval of 30 DAT were evaporated to the water phase and partitioned three times with equal volumes of dichloromethane. Afterwards, the remaining water phases of the respective plant samples were partitioned three times with equal volumes of ethyl acetate. The dichloromethane, ethyl acetate and water phases were adjusted to defined volumes with the respective solvent. Aliquots of the liquid phases were analysed by LSC measurement.
Aliquots of the homogenised extraction residue were combusted for the determination of the residual radioactive residue (RRR). To release the non-soluble radioactivity, the remaining residual radioactive residues were treated with ammonia solution (1%) and/or enzymes (cellulase, macerozyme, amylases, amyloglucosidase, laccase, hesperidinase, tyrosinase/laccase). The total radioactive residues (TRR) were obtained by combustion of sample aliquots or, alternatively, by calculating the sum of ERR and RRR values.
The identification of the metabolites is based on HPLC comparison with certified reference standards (^{14}C-labeled carbohydrates).

II. RESULTS AND DISCUSSION

A. TOTAL RADIOACTIVE RESIDUES (TRRs)

The values for the total radioactive residues are summarised in Table 6.6/2 for all the individual crop samples and in Table 6.6/3 for all the soil samples.
For all soil samples, the residue levels (TRR) were determined by direct combustion analysis of subsamples. Measurements were carried out after application (petri dishes), after soil aging and ploughing as well as after harvest of the individual mature crops for each plant back interval.
The radioactive residues in the top layer of the soil had an average concentration of 6.439 mg/ 226.8 cm^2 directly after application. The residue concentration in the top soil layer after aging and ploughing decreased from 0.295 mg/kg at 30 DAT to 0.212 mg/kg at 120 DAT and 0.179 mg/kg at 365 DAT. After harvest of the mature crops, the residue levels in soil remained more or less stable for the plant back intervals of 30, 120 and 365 DAT.

Furthermore, the total radioactive residues (TRR) were measured in immature and mature lettuce leaves, white radish top and root, and spring wheat forage, straw, hay and grain after each plant back interval (see Table 6.6/2). The calculated TRR values (ERR + RRR) were used as 100 % TRR for all further calculations.

The total radioactive residues (TRR) in lettuce (immature and mature samples) did not exceed 0.128 mg/kg for all plant back intervals. The TRR in white radish top was 0.168 mg/kg at a plant back interval of 30 DAT, decreasing to 0.019 mg/kg after 120 DAT of soil aging and to 0.003 mg/kg (TRR combusted) after 365 days of soil aging. The total radioactive residues in white radish roots decreased from 0.128 mg/kg (30 DAT), to 0.012 mg/kg (120 DAT) and finally to 0.001 mg/kg (365 DAT, TRR combusted).
In spring wheat, the highest residue levels were measured in hay (ranging from 0.070 to 1.591 mg/kg) and straw (0.049 to 1.107 mg/kg). The total radioactive residues in grain accounted for 0.041 to 0.711 mg/kg.

Table 6.6/2 Total radioactive residues in crops after treatment with ^{14}C-BAS 351 H

Crop Parts (Days After Sowing /Planting, DAP)	TRR Determined by Direct Combustion [mg/kg]	TRR Calculated* [mg/kg]
Plant back interval: 30 DAT		
Immature lettuce (40 DAP)	0.133	0.128
Mature lettuce (61 DAP)	0.079	0.076
White radish top (76 DAP)	0.169	0.168
White radish root (76 DAP)	0.138	0.128
Spring wheat forage (60 DAP)	0.279	0.270
Spring wheat hay (60 DAP)	1.712	1.591
Spring wheat straw (117 DAP)	1.337	1.107
Spring wheat grain (117 DAP)	0.732	0.711
Plant back interval: 120 DAT		
Immature lettuce (27 DAP)	0.013	0.011
Mature lettuce (41 DAP)	0.012	0.013
White radish top (77 DAP)	0.021	0.019
White radish root (77 DAP)	0.012	0.012
Spring wheat forage (56 DAP)	0.031	0.029
Spring wheat hay (56 DAP)	0.148	0.146
Spring wheat straw (103 DAP)	0.137	0.127
Spring wheat grain (103 DAP)	0.256	0.267
Plant back interval: 365 DAT		
Immature lettuce (34 DAP)	0.007	<0.01
Mature lettuce (62 DAP)	0.002	<0.01
White radish top (83 DAP)	0.003	<0.01
White radish root (83 DAP)	0.001	<0.01
Spring wheat forage (74 DAP)	0.007	<0.01
Spring wheat hay (74 DAP)	0.053	0.070
Spring wheat straw (133 DAP)	0.047	0.049
Spring wheat grain (133 DAP)	0.041	0.041

* Sum of ERR (methanol extract and water extract) and RRR (extraction residue)
DAT: Days after treatment

Table 6.6/3 Total radioactive residues in soil samples following treatment with ^{14}C-BAS 351 H

Soil Samples (Days After Treatment DAT)	TRRs [mg/kg] Determined by Direct Combustion
Plant back interval: 30 DAT	
After ploughing	
(30 DAT)	0.295
After harvest of mature crops	
Lettuce (91 DAT)	0.487
White radish (106 DAT)	0.186
Spring wheat (147 DAT)	0.217
Plant back interval: 120 DAT	
After ploughing	
(120 DAT)	0.212
After harvest of mature crops	
Lettuce (161)	0.217
White radish (197 DAT)	0.210
Spring wheat (223 DAT)	0.217
Plant back interval: 365 DAT	
After ploughing	
(365 DAT)	0.179
After harvest of mature crops	
Lettuce (427)	0.144
White radish (448 DAT)	0.153
Spring wheat (498 DAT)	0.173

DAT: Days after treatment

B. EXTRACTION AND CHARACTERISATION OF RESIDUES

1. Extraction and characterisation of residues in lettuce

Immature Lettuce

The extractability of radioactive residues from immature lettuce with methanol and water was similar for the plant back intervals of 30 and 120 DAT (ERR 30 DAT: 45.4% TRR and 120 DAT: 44.1% TRR). The major part of radioactive residues was extracted with methanol (30 DAT: 40.0% TRR and 120 DAT: 37.7% TRR), whereas only small amounts were subsequently extracted with water (30 DAT: 5.4% TRR and 120 DAT: 6.4% TRR). The residues after solvent extraction still accounted for 0.070 mg/kg or 54.6% TRR (30 DAT) and 0.006 mg/kg or 55.9% TRR (120 DAT). Thus, the residues were further investigated using a sequential solubilisation procedure. Immature lettuce of the plant back interval of 365 DAT was not extracted due to low residue level of 0.007 mg/kg.
In order to classify the radioactive residues into organosoluble and water soluble components, the methanol extract of immature lettuce (30 DAT) was partitioned between dichloromethane, ethyl acetate and water. The partitions showed that the major part of the radioactive residues extracted with methanol was water soluble (27.2% of the TRR), smaller amounts were found in the organosoluble fractions (14.1% TRR) (see Table 6.6/5).

Mature Lettuce

The extractability of radioactive residues from mature lettuce with methanol and water was comparable for the plant back intervals of 30 and 120 DAT (ERR 30 DAT: 47.3% TRR and 120 DAT: 57.6% TRR). The major part of radioactive residues was extracted with methanol (30 DAT: 42.1% TRR and 120 DAT: 50.8% TRR), whereas only small amounts were subsequently extracted with water (30 DAT: 5.2% TRR and 120 DAT: 6.8% TRR). The residues after solvent extraction still accounted for 0.040 mg/kg or 52.7% TRR (30 DAT) and 0.006 mg/kg or 42.4% TRR (120 DAT). Thus, the residues were further investigated using a sequential solubilisation procedure. Mature lettuce of the plant back interval of 365 DAT was not extracted due to low residue level of 0.002 mg/kg.
In order to classify the radioactive residues into organosoluble and water soluble components, the methanol extract of mature lettuce (30 DAT) was partitioned between dichloromethane, ethyl acetate and water. The partitions showed that the major part of the radioactive residues extracted with methanol was water soluble (28.8% of the TRR), smaller amounts were found in the organosoluble fractions (11.9% TRR) (see Table 6.6/5).

2. Extraction and characterisation of residues in white radish root and top

White radish top

The extractability of radioactive residues from white radish top with methanol and water was comparable for the plant back intervals of 30 and 120 DAT (ERR 30 DAT: 48.4% TRR and 120 DAT: 43.7% TRR). The major part of radioactive residues was extracted with methanol (30 DAT: 34.2% TRR and 120 DAT: 28.4% TRR) and another significant portion was subsequently extracted with water (30 DAT: 14.3% TRR and 120 DAT: 15.3% TRR). The residues after solvent extraction still accounted for 0.087 mg/kg or 51.6% TRR (30 DAT) and 0.010 mg/kg or 56.3% TRR (120 DAT). Thus, the residues were further investigated using a sequential solubilisation procedure. White radish top of the plant back interval of 365 DAT was not extracted due to low residue level of 0003 mg/kg.
In order to classify the radioactive residues into organosoluble and water soluble components, the methanol extract of white radish top (30 DAT) was partitioned between dichloromethane, ethyl acetate and water. The partitions showed that the major part of the radioactive residues extracted with methanol was water soluble (26.3% of the TRR), smaller amounts were found in the organosoluble fractions (8.8% TRR) (see Table 6.6/5).

White radish root

The extractability of radioactive residues from white radish root with methanol and water was somewhat higher for the plant back interval of 120 DAT as compared to 30 DAT (ERR 30 DAT: 54.2% TRR and 120 DAT: 71.3% TRR). The major part of radioactive residues was extracted with methanol (30 DAT: 50.0% TRR and 120 DAT: 67.8% TRR), whereas only minor amounts were subsequently extracted with water (30 DAT: 4.2% TRR and 120 DAT: 3.5% TRR). The residues after solvent extraction still accounted for 0.059 mg/kg or 45.8% TRR (30 DAT) and 0.003 mg/kg or 28.7% TRR (120 DAT). Thus, the residues were further investigated using a sequential solubilisation procedure. White radish root of the plant back interval of 365 DAT was not extracted due to low residue level of 0.001 mg/kg.
In order to classify the radioactive residues into organosoluble and water soluble components, the methanol extract of white radish root (30 DAT) was partitioned between dichloromethane, ethyl acetate and water. The partitions showed that the major part of the radioactive residues extracted with methanol was water soluble (46.4% of the TRR), smaller amounts were found in the organosoluble fractions (3.3% TRR) (see Table 6.6/5).

3. Extraction and characterisation of residues in spring wheat

Spring wheat forage

The extractability of radioactive residues from spring wheat forage with methanol and water was similar for the plant back intervals of 30 and 120 DAT (ERR 30 DAT: 28.4% TRR and 120 DAT: 27.5% TRR). The major part of radioactive residues was extracted with methanol (30 DAT: 24.3% TRR and 120 DAT: 23.8% TRR), whereas only minor amounts were extracted with water (30 DAT: 4.1% TRR and 120 DAT: 3.7% TRR). The residues after solvent extraction still accounted for 0.193 mg/kg or 71.6% TRR (30 DAT) and 0.021 mg/kg or 72.5% TRR (120 DAT). Thus, the residues were further investigated using a sequential solubilisation procedure. Spring wheat forage of the plant back interval of 365 DAT was not extracted due to low residue level of 0.007 mg/kg.
In order to classify the radioactive residues into organosoluble and water soluble components, the methanol extract of spring wheat forage (30 DAT) was partitioned between dichloromethane, ethyl acetate and water. The partitions showed that a greater part of the radioactive residues extracted with methanol was water soluble (13.9% of the TRR), smaller amounts were found in the organosoluble fractions (9.3% TRR) (see Table 6.6/5).

Spring wheat hay

The extractability of radioactive residues from spring wheat hay with methanol and water was comparable for the plant back intervals of 30, 120 and 365 DAT (ERR 30 DAT: 24.7% TRR, 120 DAT: 22.7% TRR and 365 DAT: 25.2% TRR). The major part of radioactive residues was extracted with methanol (30 DAT: 16.6% TRR, 120 DAT: 15.0% TRR and 365 DAT: 19.0% TRR) and another portion subsequently with water (30 DAT: 8.2% TRR, 120 DAT: 7.7% TRR and 365 DAT: 6.2% TRR). The residues after solvent extraction still accounted for 1.198 mg/kg or 75.3% TRR (30 DAT), 0.113 mg/kg or 77.3% TRR (120 DAT) and 0.052 mg/kg and 74.8% TRR (365 DAT). Thus, the residues of the plant back intervals of 30 DAT and 120 DAT were further investigated using a sequential solubilisation procedure.
In order to classify the radioactive residues into organosoluble and water soluble components, the methanol extract of spring wheat hay (30 DAT) was partitioned between dichloromethane, ethyl acetate and water. The partitions showed that similar portions of the radioactive residues extracted with methanol were organosoluble and water soluble (7.1% TRR (sum organosoluble) and 9.8% TRR, respectively) (see Table 6.6/5).

Spring wheat straw

The extractability of radioactive residues from spring wheat straw with methanol and water was comparable for the plant back intervals of 30, 120 and 365 DAT (ERR 30 DAT: 23.7% TRR), 120 DAT: 22.4% TRR and 365 DAT: 30.0% TRR). The major part of radioactive residues was extracted with methanol (30 DAT: 15.5% TRR, 120 DAT: 15.6% TRR and 365 DAT: 21.5% TRR) and another portion subsequently with water (30 DAT: 8.2% TRR, 120 DAT: 6.8% TRR and 365 DAT: 8.6% TRR). The residues after solvent extraction still accounted for 0.845 mg/kg or 76.3% TRR (30 DAT), 0.099 mg/kg or 77.6% TRR (120 DAT) and 0.034 mg/kg and 70.0% TRR (365 DAT). Thus, the residues of the plant back intervals of 30 DAT and 120 DAT were further investigated using a sequential solubilisation procedure.
In order to classify the radioactive residues into organosoluble and water soluble components, the methanol extract of spring wheat straw (30 DAT) was partitioned between dichloromethane, ethyl acetate and water. The partitions showed that similar portions of the radioactive residues extracted with methanol were organosoluble and water soluble (7.6% TRR (sum organosoluble) and 7.5% TRR, respectively) (see Table 6.6/5).

Spring wheat grain

The extractability of radioactive residues from spring wheat grain with methanol and water was comparable for the plant back intervals of 30, 120 and 365 DAT (ERR 30 DAT: 10.8% TRR, 120 DAT: 8.5% TRR and 365 DAT: 14.0% TRR). One part of radioactive residues was extracted with methanol (30 DAT: 5.0% TRR, 120 DAT: 4.3% TRR and 365 DAT: 5.9% TRR) and a comparable portion subsequently with water (30 DAT: 5.8% TRR, 120 DAT: 4.2% TRR and 365 DAT: 8.1% TRR). The residues after solvent extraction still accounted for 0.634 mg/kg or 89.2% TRR (30 DAT), 0.244 mg/kg or 91.5% TRR (120 DAT) and 0.035 mg/kg and 86.0% TRR (365 DAT). Thus, the residues of the plant back intervals of 30 DAT and 120 DAT were further investigated using a sequential solubilisation procedure.
In order to classify the radioactive residues into organosoluble and water soluble components, the methanol extract of spring wheat grain (30 DAT) was partitioned between dichloromethane, ethyl acetate and water. The partitions showed that similar portions of the radioactive residues extracted with methanol were organosoluble and water soluble (2.1% TRR (sum organosoluble) and 2.4% TRR, respectively) (see Table 6.6/5).

Table 6.6/4 Extractability of radioactive residues in rotational crop after ^{14}C-BAS 351 H treatment after plant back intervals of 30, 120 and 365 days

Crop Parts (Days After Sowing/Planting, DAP)	TRR[1]	Methanol Extract		Water Extract		ERR[2]		RRR[3]	
	[mg/kg]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Plant back interval: 30 DAT									
Immature lettuce (40)	0.128	0.051	40.0	0.007	5.4	0.058	45.4	0.070	54.6
Mature lettuce (61)	0.076	0.032	42.1	0.004	5.2	0.036	47.3	0.040	52.7
White radish top (76)	0.168	0.057	34.2	0.024	14.3	0.081	48.4	0.087	51.6
White radish root (76)	0.128	0.064	50.0	0.005	4.2	0.070	54.2	0.059	45.8
Spring wheat forage (60)	0.270	0.065	24.3	0.011	4.1	0.077	28.4	0.193	71.6
Spring wheat hay (60)	1.591	0.263	16.6	0.130	8.2	0.393	24.7	1.198	75.3
Spring wheat straw (117)	1.107	0.172	15.5	0.090	8.2	0.262	23.7	0.845	76.3
Spring wheat straw* (117)	1.107	0.178	16.0	-	-	-	-	-	-
Spring wheat grain (117)	0.711	0.035	5.0	0.041	5.8	0.077	10.8	0.634	89.2
Spring wheat grain* (117)	0.711	0.027	3.8	-	-	-	-	-	-
Plant back interval: 120 DAT									
Immature lettuce (27)	0.011	0.004	37.7	0.001	6.4	0.005	44.1	0.006	55.9
Mature lettuce (41)	0.013	0.007	50.8	0.001	6.8	0.008	57.6	0.006	42.4
White radish top (77)	0.019	0.005	28.4	0.003	15.3	0.008	43.7	0.010	56.3
White radish root (77)	0.012	0.008	67.8	0.000	3.5	0.009	71.3	0.003	28.7
Spring wheat forage (56)	0.029	0.007	23.8	0.001	3.7	0.008	27.5	0.021	72.5
Spring wheat hay (56)	0.146	0.022	15.0	0.011	7.7	0.033	22.7	0.113	77.3
Spring wheat straw (103)	0.127	0.020	15.6	0.009	6.8	0.028	22.4	0.099	77.6
Spring wheat grain (103)	0.267	0.011	4.3	0.011	4.2	0.023	8.5	0.244	91.5
Plant back interval: 365 DAT									
Spring wheat hay (74)	0.070	0.013	19.0	0.004	6.2	0.018	25.2	0.052	74.8
Spring wheat straw (133)	0.049	0.011	21.5	0.004	8.6	0.015	30.0	0.034	70.0
Spring wheat grain (133)	0.041	0.002	5.9	0.003	8.1	0.006	14.0	0.035	86.0

[1] TRR = sum of ERR and RRR
[2] ERR = extractable radioactive residue (methanol extract and water extract),
[3] RRR = residual radioactive residue
* Extraction 2; data for water extract 2, ERR and RRR are not shown because they are of no relevance

Table 6.6/5 Partition characteristics of radioactive residues extracted with methanol from rotational crop samples after ^{14}C-BAS 351 H treatment and plant back intervals of 30 days

Crop Parts	DAT[1)]	Methanol Extract		Organosoluble				Organo-soluble Sum		Water Soluble		Reco-very[2)]
				Dichloro-methane		Ethyl Acetate						
		[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[%]
Plant back interval: 30 DAT												
Immature lettuce	30	0.051	40.0	0.015	11.4	0.003	2.7	0.018	14.1	0.035	27.2	103.4
Mature lettuce		0.032	42.1	0.008	9.9	0.002	2.1	0.009	11.9	0.022	28.8	96.7
White radish top		0.057	34.2	0.011	6.7	0004	2.1	0.015	8.8	0.044	26.3	102.8
White radish root		0.064	50.0	0.002	1.8	0.002	1.4	0.004	3.3	0.060	46.4	99.2
Spring wheat forage		0.065	24.3	0.020	7.4	0.005	1.8	0.025	9.3	0.038	13.9	95.4
Spring wheat hay		0.263	16.6	0.099	6.2	0.014	0.9	0.113	7.1	0.156	9.8	101.9
Spring wheat straw		0.172	15.5	0.063	5.7	0.021	1.9	0.084	7.6	0.083	7.5	97.2
Spring wheat grain		0.035	5.0	0.012	1.7	0.003	0.4	0.015	2.1	0.017	2.4	91.0

[1)] DAT = Days after treatment
[2)] Recovery calculated as (dichloromethane + ethyl acetate + water soluble) [mg/kg] * 100 / methanol extract [mg/kg]

Table 6.6/6 Quantitative distribution of the non-released radioactivity in rotational crops after treatment with ^{14}C-BAS 351 H

Fraction / Supernatant	Crop Parts							
	Immature lettuce (40 DAP[1)])		Matture lettuce (61 DAP)		White radish Top (76 DAP)		White radish root (76 DAP)	
	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]	[mg/kg]	[% TRR]
Plant back interval: 30 DAT[2)]								
RRR	0.070	54.6	0.040	52.7	0.087	51.6	0.059	45.8
NH_4OH Solubilisate	0.004	2.8	0.002	2.9	0.005	2.9	0.003	2.6
Macerozyme / Cellulase Solubilisate[3)]	0.018	14.1	0.016	21.2	0.035	21.1	0.027	20.7
Glucosidase / Hesperidinase Solubilisate	0.0049	3.8	0.004	5.4	0.007	4.1	0.009	6.8
α-Amylase / β -Amylase / Amyloglucosidase Solubilisate	0.0043	3.3	0.001	1.4	0.004	2.1	0.003	2.2
Sum of Solubilised Radioactive Residues	0.031	24.0	0.024	30.9	0.051	30.1	0.041	32.3
Final Residue	0.023	17.6	0.018	23.4	0.028	16.8	0.016	12.5
Procedural Recovery [%][4)]	76.3		103.2		91.0		97.8	
Plant back interval: 120 DAT								
RRR	0.006	55.9	0.006	42.4	0.010	56.3	0.003	28.7
NH_4OH Solubilisate	0.001	5.0	0.000	3.7	0.001	4.2	0.000	1.4
Macerozyme Solubilisate	0.001	12.4	0.001	10.1	0.002	12.7	0.001	7.8
Glucosidase / Hesperidinase Solubilisate	n.a.		n.a.		0.001	2.8	0.000	1.8
Sum of Solubilised Radioactive Residues	0.002	17.4	0.002	13.8	0.004	19.6	0.001	10.9
Final Residue	0.004	38.4	0.003	23.6	0.005	26.1	0.002	14.3
Procedural Recovery [%]	99.8		88.2		81.3		88.0	

[1)] DAP = Days after planting (or sowing, respectively)
[2)] DAT = Days after treatment
[3)] Cellulase was additionally applied in the case of mature lettuce, white radish top and root
[4)] Recovery calculated as (sum of solubilised radioactive residues + final residue) [mg/kg] • 100 / RRR [mg/kg]
n.a. = not applied

Table 6.6/7 Quantitative distribution of the non-released radioactivity in rotational crops after treatment with ^{14}C-BAS 351 H

Fraction / Supernatant	Crop Parts							
	Spring Wheat Forage (60 DAP[1])		Spring Wheat Hay (60 DAP)		Spring Wheat Straw (117 DAP)		Spring Wheat Grain (117 DAP)	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Plant back interval: 30 DAT[2]								
RRR	0.193	71.6	1.198	75.3	0.845	76.3	0.634	89.2
NH_4OH Solubilisate	0.009	3.3	0.091	5.7	0.028	2.5	0.072	10.1
Macerozyme / Cellulase Solubilisate [3) 4)]	0.056	20.6	0.134	8.4	0.085	7.7	0.201	28.2
Glucosidase / Hesperidinase Solubilisate	0.013	5.0	0.039	2.5	0.033	3.0	0.061	8.6
α-Amylase / β -Amylase / Amyloglucosidase Solubilisate	0.002	0.9	0.019	1.2	0.012	1.1	0.152	21.3
Tyrosinase / Laccase Solubilisate	0.001	0.4	n.a.		0.018	1.6	0.002	0.2
Microwave Incubation	0.008	2.8	0.049	3.1	0.155	14.0	0.025	3.5
6 N HCl	0.025	9.3	0.273	17.2	0.154	14.0	n.a.	
2 N NaOH	0.020	7.4	0.180	11.3	0.159	14.4	n.a.	
Sum of Solubilised Radioactive Residues	0.134	49.8	0.785	49.3	0.645	58.3	0.511	71.9
Final Residue	0.011	4.2	0.068	4.3	0.035	3.1	0.041	5.7
Procedural Recovery [%][5]	75.4		71.2		80.4		87.1	
Plant back interval: 120 DAT								
RRR	0.021	72.5	0.113	77.3	0.099	77.6	0.244	91.5
NH_4OH Solubilisate	0.001	3.5	0.008	5.3	0.005	3.7	0.019	7.3
Macerozyme / Cellulase Solubilisate [3)]	0.005	18.1	0.016	11.0	0.006	4.8	0.114	42.9
Glucosidase / Hesperidinase Solubilisate	0.001	4.4	0.004	2.8	0.002	1.6	0.044	16.4
α-Amyase / β -Amylase / Amyloglucosidase Solubilisate	0.001	2.2	0.002	1.1	0.001	1.1	0.012	4.4
Microwave Incubation	0.001	2.2	0.005	3.6	0.005	3.9	n.a.	
Sum of Solubilised Radioactive Residues	0.009	30.4	0.035	23.9	0.019	15.0	0.190	71.1
Final Residue	0.008	25.9	0.064	44.1	0.071	55.8	0.021	7.9
Procedural Recovery [%][5]	77.7		87.9		91.2		86.3	

1) DAP = Days after planting (or sowing, respectively)
2) DAT = Days after treatment
3) Cellulase was additionally applied in the case of spring wheat forage, straw and grain
4) In the work-up of spring wheat grain (30 DAT) the enzymatic solubilisation procedures applying macerozyme / cellulase and glucosidase / hesperidinase were applied in a reversed order
5) Recovery calculated as (sum of solubilised radioactive residues + final residue) [mg/kg] • 100 / RRR [mg/kg]

n.a. = not applied

C. IDENTIFICATION, CHARACTERISATION AND QUANTITATION OF RESIDUES

Quantitative summaries of parent, metabolite and characterised fractions in lettuce, white radish and spring wheat are given in Table 6.6/8 - Table 6.6/11.

1. Identification, characterisation and quantitation of extractable residues in lettuce head

Immature lettuce

30 DAT: The analysis of the methanol extractable residues of immature lettuce (30 DAT) using HPLC method LC14 resulted in one main polar peak (0.0303 mg/kg or 23.7% TRR) and further minor constituents amongst the parent compound BAS 351 H (0.0013 mg/kg or 1.0% TRR). The remaining minor constituents were characterised by their chromatographic properties (each below or equal to 0.0054 mg/kg or 4.2% TRR). The metabolic pattern of the same sample was confirmed using HPLC method LC26.
For further characterisation of the polar fraction, the methanol extract was additionally analysed using HPLC method LC21, specific for saccharides. The analysis resulted in a pattern of nine peaks, whereby fructose / glucose were assigned to the main peak (0.018 mg/kg or 14.2% TRR) and sucrose to 0.005 mg/kg or 4.2% TRR. Therefore, the polar fraction was composed of significant portions of carbohydrates. The presence of carbohydrates was confirmed using HPLC method LC29 with a monosaccharide column.
Analysis of the concentrated water extract using HPLC method LC14 showed one peak (0.0068 mg/kg or 5.3%TRR), which was identified as polar fraction containing significant portions of carbohydrates as main components. HPLC analysis of the same extract using method LC26 confirmed the identity of the polar fraction.
In total, 30.0% of the TRR were identified as polar fraction (carbohydrates) and BAS 351 H, and additional 14.0% of the TRR were characterised by their HPLC elution behaviour.
In order to investigate the residual radioactive residues (RRR), the residues remaining after extraction of immature lettuce with methanol and water (0.070 mg/kg or 54.6% TRR) were subjected to a sequential solubilisation procedure. Together, 0.031 mg/kg or 24.0% TRR were released during the sequential solubilisation procedure.

In total, 44.0% of the TRR were identified or characterised from the ERR and additional 24.0% of the TRR were identified or characterised after solubilisation from the RRR.

120 DAT: HPLC analysis of the methanol extract of immature lettuce using method LC14 resulted in one peak (0.004 mg/kg or 37.7% TRR), which was identified as polar fraction. In addition, 0.001 mg/kg or 6.4% TRR were characterised by their extractability with water. The polar peak was confirmed in the same extract using HPLC method LC26.
In total, 37.7% of the TRR were identified as polar fraction (carbohydrates), and additional 6.4% of the TRR were characterised by their extractability with water.

In order to investigate the residual radioactive residues (RRR), the residues remaining after extraction of immature lettuce with methanol and water (0.006 mg/kg or 55.9% TRR) were subjected to a sequential solubilisation procedure. Together, 0.002 mg/kg or 17.4% TRR were released during the sequential solubilisation procedure.

In total, 44.1% of the TRR were identified or characterised from the ERR and additional 17.4% of the TRR were characterised by solubilisation from the RRR.

Mature lettuce

30 DAT: The analysis of the methanol extractable residues of immature lettuce (30 DAT) using HPLC method LC14 yielded a pattern of six peaks of which the main component was characterised as polar fraction (0.0259 mg/kg or 34.0% TRR). The unchanged parent compound was identified with 0.0010 mg/kg or 1.2% TRR. The remaining constituents were characterised by their chromatographic properties (0.0013 mg/kg or 1.7% TRR). HPLC analysis of a similar extract using HPLC method LC26 confirmed the peak assignment.
For further characterisation of the polar fraction the extractable residues were investigated by analysis of the methanol extract 2 using HPLC method LC21, which is suitable for saccharide separation. The analysis resulted in a pattern of nine peaks, whereby fructose / glucose were assigned to the main peak with 0.0145 mg/kg or 19.3% TRR and sucrose to 0.0014 mg/kg or 1.8% TRR. The presence of carbohydrates was confirmed using HPLC method LC29 with a monosaccharide column. The assignment of the components was based on co-chromatography experiments with the same methanol extract and the reference items fructose, glucose and sucrose. The additional investigation of the methanol extractable residues suggested that the polar fraction was composed of significant portions of carbohydrates.
Analysis of the concentrated water extract using HPLC method LC14 resulted in one peak with 0.004 mg/kg or 4.6% TRR, which was identified as polar fraction containing significant portions of carbohydrates as main components. HPLC analysis of the same extract using method LC26 confirmed the identity of the polar fraction.
In total, 39.8% of the TRR were identified as polar fraction (carbohydrates) and the unchanged parent compound BAS 351 H. Additional 3.5% of the TRR were characterised by their HPLC elution behaviour.
In order to investigate the residual radioactive residues (RRR), the residues remaining after extraction of immature lettuce with methanol and water (0.040 mg/kg or 52.7% TRR) were subjected to a sequential solubilisation procedure. Together, 0.024 mg/kg or 30.9% TRR were released during the sequential solubilisation procedure.

In total, 43.3% of the TRR were identified or characterised from the ERR and additional 30.9% of the TRR were identified or characterised after solubilisation from the RRR.

120 DAT: HPLC analysis of the methanol extract of mature lettuce using method LC14 resulted in a pattern of five peaks, of which the peak with 0.0045 mg/kg or 34.0% TRR was the main component and identified as polar fraction. The remaining constituents were characterised by their chromatographic properties (each below or equal to 0.0013 mg/kg or 10.2% TRR). In addition, 0.0009 mg/kg or 6.8% TRR were characterised by their extractability with water. The metabolic pattern was confirmed in the same extract using HPLC method LC26.
In total, 36.5% of the TRR were identified as polar fraction (carbohydrates) and the unchanged parent compound BAS 351 H. Additional 14.3% of the TRR were characterised by their HPLC elution behavior. Further 6.8% of the TRR were characterised by their extractability with water.
In order to investigate the residual radioactive residues (RRR), the residues remaining after extraction of immature lettuce with methanol and water (0.006 mg/kg or 42.4% TRR) were subjected to a sequential solubilisation procedure. Together, 0.002 mg/kg or 13.8% TRR were released during the sequential solubilisation procedure.

In total, 57.6% of the TRR were identified or characterised from the ERR and additional 13.8% of the TRR were characterised by solubilisation from the RRR.

Table 6.6/8 Total identified, characterised and final radioactive residues in lettuce after treatment with ^{14}C-BAS 351 H

Metabolite / Fraction	Immature Lettuce Leaves [mg/kg]	Immature Lettuce Leaves [%TRR]	Mature Lettuce Leaves [mg/kg]	Mature Lettuce Leaves [%TRR]
Plant back interval: 30 DAT				
Total radioactive residue (TRR)	0.128	100.0	0.076	100.0
Extractable radioactive residue (ERR)	0.058	45.4	0.036	47.3
Total identified from ERR	0.038	30.0	0.030	39.8
Total characterised from ERR	0.018	14.0	0.003	3.5
Total identified or characterised from ERR [1)]	**0.056**	**44.0**	**0.033**	**43.3**
Residual radioactive residue (after solvent extraction, RRR)	0.070	54.6	0.040	52.7
Total Identified Radioactive Residues Solubilised from RRR	0.027	20.7	0.023	29.6
Total Characterised Radioactive Residues Solubilised from RRR	0.004	3.3	0.001	1.4
Total identified or characterised radioactive residues solubilised from RRR [2)]	**0.031**	**24.0**	**0 024**	**30.9**
Total identified	**0.065**	**50.7**	**0.053**	**69.4**
Total characterised	**0.022**	**17.4**	**0.004**	**4.9**
Total identified or characterised	**0.087**	**68.0**	**0.057**	**74.3**
Final residue[2)]	**0.023**	**17.6**	**0.018**	**23.4**
Grand Total	**0.110**	**85.6**	**0.074**	**97.7**
Plant back interval: 120 DAT				
Total radioactive residue (TRR)	0.011	100.0	0.013	100.0
Extractable radioactive residue (ERR)	0.005	44.1	0.008	57.6
Total identified from ERR	0.004	37.7	0.005	36.5
Total characterised from ERR	0.001	6.4	0.003	21.1
Total identified or characterised from ERR [1)]	**0.005**	**44.1**	**0.008**	**57.6**
Residual radioactive residue (after solvent extraction, RRR)	0.006	55.9	0.006	42.4
Total Characterised Radioactive Residues Solubilised from RRR	0.002	17.4	0.002	13.8
Total characterised radioactive residues solubilised from RRR [2)]	**0.002**	**17.4**	**0.002**	**13.8**
Total identified	**0.004**	**37.7**	**0.005**	**36.5**
Total characterised	**0.003**	**23.8**	**0.005**	**34.9**
Total identified or characterised	**0.007**	**61.5**	**0.010**	**71.4**
Final residue	**0.004**	**38.4**	**0.003**	**23.6**
Grand Total	**0.011**	**99.9**	**0.013**	**95.0**

[1)] ERR = Extractable Radioactive Residue (methanol extract and water extract)
[2)] see Table 6.6/6

2. Identification, characterisation and quantitation of extractable residues in white radish roots and tops

White radish top

30 DAT: The analysis of the methanol extractable residues of white radish top (30 DAT) using HPLC method LC14 yielded a pattern of four peaks, of which the main component was characterised as polar fraction (0.0481 mg/kg or 28.7% TRR). The remaining constituents were characterised by their chromatographic properties (each below or equal to 0.0029 mg/kg or 1.7% TRR). Analysis of the same extract using HPLC method LC26 confirmed the metabolic pattern. For further characterisation of the polar fraction the methanol extract was additionally analysed using HPLC method LC21. The analysis resulted in a pattern of nine peaks, of which the main component was assigned to fructose / glucose / sucrose (0.029 mg/kg or 17.2% TRR). Therefore, the polar fraction was composed of significant portions of carbohydrates. The presence of carbohydrates was confirmed using HPLC method LC29 with a monosaccharide column.
Analysis of the concentrated water extract using HPLC method LC14 resulted in one peak, which was identified as polar fraction (0.0231 mg/kg or 13.7% TRR) containing significant portions of carbohydrates as main components. HPLC analysis of an equivalent sample using method LC26 confirmed the polar peak.
In total, 42.4% of the TRR were identified as polar fraction (carbohydrates), and additional 3.9% of the TRR were characterised by their HPLC elution behaviour.

In order to investigate the residual radioactive residues (RRR), the residues remaining after extraction of white radish top with methanol and water (0.087 mg/kg or 51.6% TRR) were subjected to a sequential solubilisation procedure. Together, 0.051 mg/kg or 30.1% TRR were released during the sequential solubilisation procedure.

In total, 46.3% of the TRR were identified or characterised from the ERR and additional 30.1% of the TRR were characterised by solubilisation from the RRR.

120 DAT: Analysis of the methanol extract of white radish top using HPLC method LC14 resulted in one peak (0.005 mg/kg or 28.4% TRR), which was identified as polar fraction. In addition, 0.003 mg/kg or 15.3% TRR were characterised by their extractability with water. The polar peak was confirmed upon analysis of the same extract using HPLC method LC26.
In total, 28.4% of the TRR were assigned as polar fraction (carbohydrates), and additional 15.3% of the TRR were characterised by their extractability with water.

In order to investigate the residual radioactive residues (RRR), the residues remaining after extraction of white radish top with methanol and water (0.010 mg/kg or 56.3% TRR) were subjected to a sequential solubilisation procedure. Together, 0.004 mg/kg or 19.6% TRR were released during the sequential solubilisation procedure.

In total, 43.7% of the TRR were identified or characterised from the ERR and additional 19.6% of the TRR were characterised by solubilisation from the RRR.

Table 6.6/9 Total identified, characterised and final radioactive residues in white radish after treatment with ^{14}C-BAS 351 H

Metabolite / Fraction	White Radish Top		White Radish Root	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Plant back interval: 30 DAT				
Total radioactive residue (TRR)	0.168	100.0	0.128	100.0
Extractable radioactive residue (ERR)	0.081	48.4	0.070	54.2
Total identified from ERR	0.071	42.4	0.066	51.7
Total characterised from ERR	0.006	3.9	0.000	0.0
Total identified or characterised from ERR [1)]	**0.078**	**46.3**	**0.066**	**51.7**
Residual radioactive residue (after solvent extraction, RRR)	0.087	51.6	0.059	45.8
Total Identified Radioactive Residues Solubilised from RRR	0.051	30.1	0.038	29.7
Total Characterised Radioactive Residues Solubilised from RRR	0.000	0.0	0.003	2.6
Total identified or characterised radioactive residues solubilised from RRR [2)]	**0.051**	**30.1**	**0.041**	**32.3**
Total identified	**0.122**	**72.5**	**0.105**	**81.4**
Total characterised	**0.006**	**3.9**	**0.003**	**2.6**
Total identified or characterised	**0.128**	**76.4**	**0.108**	**84.0**
Final residue[2)]	**0.028**	**16.8**	**0.016**	**12.5**
Grand Total	**0.157**	**93.2**	**0.124**	**96.5**
Plant back interval: 120 DAT				
Total radioactive residue (TRR)	0.019	100.0	0.012	100.0
Extractable radioactive residue (ERR)	0.008	43.7	0.009	71.3
Total identified from ERR	0.005	28.4	0.008	67.8
Total characterised from ERR	0.003	15.3	0.000	3.5
Total identified or characterised from ERR [1)]	**0.008**	**43.7**	**0.009**	**71.3**
Residual radioactive residue (after solvent extraction, RRR)	0.010	56.3	0.003	28.7
Total Characterised Radioactive Residues Solubilised from RRR	0.004	19.6	0.001	10.9
Total characterised radioactive residues solubilised from RRR [2)]	**0.004**	**19.6**	**0.001**	**10.9**
Total identified	**0.005**	**28.4**	**0.008**	**67.8**
Total characterised	**0.007**	**34.9**	**0.001**	**14.4**
Total identified or characterised	**0.012**	**63.3**	**0.010**	**82.2**
Final residue	**0.005**	**26.1**	**0.002**	**14.3**
Grand Total	**0.017**	**89.4**	**0.011**	**96.6**

[1)] ERR = Extractable Radioactive Residue (methanol extract and water extract)
[2)] see Table 6.6/6

3. Identification, characterisation and quantitation of extractable residues in wheat straw, forage, chaff and grain

Spring wheat forage

30 DAT: HPLC analysis of the concentrated methanol extract of spring wheat forage using method HPLC LC14 resulted in a pattern of three peaks. The main peak was identified as polar fraction (0.045 mg/kg or 16.6% TRR). The remaining constituents were characterised by their chromatographic properties (each below or equal to 0.008 mg/kg or 2.9% TRR). The metabolic pattern was largely confirmed upon analysis of an equivalent sample using HPLC method LC26. For further characterisation of the polar fraction the methanol extract was additionally analysed using HPLC method LC21, which resulted in a pattern of seven peaks. The main peak was assigned to fructose / glucose / sucrose (0.035 mg/kg or 13.0% TRR). Thus, the polar fraction was composed of significant portions of carbohydrates. The presence of carbohydrates was confirmed using HPLC method LC29 with a monosaccharide column.
Analysis of the concentrated water extract using HPLC method LC14 resulted in one peak, which was identified as polar fraction (0.009 mg/kg or 3.2% TRR) containing significant portions of carbohydrates as main components. HPLC analysis of the same extract using method LC26 confirmed the identity of the polar fraction.
In total, 19.8% of the TRR were identified as polar fraction (carbohydrates), and additional 5.7% of the TRR were characterised by their HPLC elution behaviour.

In order to investigate the residual radioactive residues (RRR), the residues remaining after extraction of spring wheat forage with methanol and water (0.193 mg/kg or 71.6% TRR) were subjected to a sequential solubilisation procedure. Together, 0.134 mg/kg or 49.8% TRR were released during the sequential solubilisation procedure.

In total, 25.5% of the TRR were identified or characterised from the ERR and additional 49.8% of the TRR were characterised by solubilisation from the RRR.

120 DAT: Analysis of the methanol extract of spring wheat forage using HPLC method LC14 resulted in one peak with 0.007 mg/kg or 23.8% TRR, which was identified as polar fraction. In addition, 0.001 mg/kg or 3.7% TRR were characterised by their extractability with water. HPLC analysis of the same extract using method LC26 confirmed the polar peak.
In total, 23.8% of the TRR were identified as polar fraction (carbohydrates), and additional 3.7% of the TRR were characterised by their extractability with water.

In order to investigate the residual radioactive residues (RRR), the residues remaining after extraction of spring wheat forage with methanol and water (0.021 mg/kg or 72.5% TRR) were subjected to a sequential solubilisation procedure. Together, 0.009 mg/kg or 30.4% TRR were released during the sequential solubilisation procedure.

In total, 27.5% of the TRR were identified or characterised from the ERR and additional 30.4% of the TRR were characterised by solubilisation from the RRR.

Spring wheat hay

30 DAT: HPLC analysis of the concentrated methanol extract of spring wheat hay using method LC14 yielded two peaks, of which the main peak was identified as polar fraction (0.225 mg/kg or 14.1% TRR). The remaining peak was characterised by its chromatographic properties (0.039 mg/kg or 2.5% TRR). Analysis of the same extract using HPLC method LC26 confirmed the metabolic pattern. For further characterisation of the polar fraction, the methanol extract was additionally analysed using HPLC method LC21. The main component was assigned to fructose / glucose / sucrose (0.111 mg/kg or 7.0% TRR). Thus, the polar fraction was composed of significant portions of carbohydrates. The presence of carbohydrates was confirmed using HPLC method LC29 with a monosaccharide column.
Analysis of the concentrated water extract of spring wheat hay using HPLC method LC14 resulted in the same metabolic pattern. The main constituent was identified as polar fraction (0.117 mg/kg or 7.4% TRR) containing significant portions of carbohydrates as main components.
The remaining constituents were characterised by its chromatographic properties (0.006 mg/kg or 0.4% TRR). The metabolic pattern was confirmed upon analysis of the same extract using HPLC method LC26.
In total, 21.5% of the TRR were identified as polar fraction (carbohydrates), and additional 2.8% of the TRR were characterised by their HPLC elution behaviour.

In order to investigate the residual radioactive residues (RRR), the residues remaining after extraction of spring wheat hay with methanol and water (1.198 mg/kg or 75.3% TRR) were subjected to a sequential solubilisation procedure. Together, 0.785 mg/kg or 49.3% TRR were released during the sequential solubilisation procedure.

In total, 24.3% of the TRR were identified or characterised from the ERR and additional 49.3% of the TRR were characterised by solubilisation from the RRR.

120 DAT: HPLC analysis of the methanol extract of spring wheat hay using method LC14 resulted in one peak (0.022 mg/kg or 15.0%), which was identified as polar fraction. In addition, 0.011 mg/kg or 7.7% TRR were characterised by their extractability with water. Analysis of the same extract using HPLC method LC26 confirmed the polar fraction.
In total, 15.0% of the TRR were identified as polar fraction (carbohydrates), and additional 7.7% of the TRR were characterised by their extractability with water.

In order to investigate the residual radioactive residues (RRR), the residues remaining after extraction of spring wheat hay with methanol and water (0.113 mg/kg or 77.3% TRR) were subjected to a sequential solubilisation procedure. Together, 0.035 mg/kg or 23.9% TRR were released during the sequential solubilisation procedure.

In total, 22.7% of the TRR were identified or characterised from the ERR and additional 23.9% of the TRR were characterised by solubilisation from the RRR.

365 DAT: HPLC analysis of the methanol extract of spring wheat hay using method LC14 resulted in one peak (0.013 mg/kg or 19.0% TRR), which was identified as polar fraction. In addition, 0.004 mg/kg or 6.2% TRR were characterised by their extractability with water. Analysis of the same extract using HPLC method LC26 confirmed the polar peak.
In total, 19.0% of the TRR were identified as polar fraction (carbohydrates), and additional 6.2% of the TRR were characterised by their extractability with water.

Spring wheat straw

30 DAT: Analysis of the concentrated methanol extract of spring wheat straw using HPLC method LC14 yielded a pattern of four peaks, of which the main peak was identified as polar fraction (0.103 mg/kg or 9.3% TRR). The remaining constituents were characterised by their chromatographic properties (each below or equal to 0.032 mg/kg or 2.9% TRR). HPLC analysis of the same extract using HPLC method LC26 largely confirmed the metabolic pattern. For further characterisation of the polar fraction, the methanol extract was additionally analysed using HPLC method LC21. The analysis lead to a metabolic pattern of six peaks, whereby two peaks were together assigned to fructose / glucose / sucrose (0.021 mg/kg or 1.9% TRR and 0.012 mg/kg or 1.1% TRR, respectively). Therefore, the polar fraction was composed of significant portions of carbohydrates. The presence of carbohydrates was confirmed using HPLC method LC29 with a monosaccharide column.
HPLC analysis of the concentrated water extract of spring wheat straw using method LC14 showed one peak, which was identified as polar fraction (0.082 mg/kg or 7.4% TRR) containing significant portions of carbohydrates as main components. Analysis of the same extract using HPLC method LC26 confirmed the polar peak.
In total, 16.8% of the TRR were identified as polar fraction (carbohydrates), and additional 5.9% of the TRR were characterised by their HPLC elution behaviour.

In order to investigate the residual radioactive residues (RRR), the residues remaining after extraction of spring wheat hay with methanol and water (0.845 mg/kg or 76.3% TRR) were subjected to a sequential solubilisation procedure. Together, 0.645 mg/kg or 58.3% TRR were released during the sequential solubilisation procedure.

In total, 22.7% of the TRR were identified or characterised from the ERR and additional 58.3% of the TRR were characterised by solubilisation from the RRR.

120 DAT: Analysis of the methanol extract of spring wheat straw using HPLC method LC14 resulted in one peak (0.020 mg/kg or 15.6% TRR), which was identified as polar fraction. In addition, 0.009 mg/kg or 6.8% TRR were characterised by their extractability with water. The polar peak was confirmed upon analysis of the same extract using HPLC method LC26.
In total, 15.6% of the TRR were assigned as polar fraction (carbohydrates), and additional 6.8% of the TRR were characterised by their extractability with water.

In order to investigate the residual radioactive residues (RRR), the residues remaining after extraction of spring wheat hay with methanol and water (0.099 mg/kg or 77.6% TRR) were subjected to a sequential solubilisation procedure. Together, 0.019 mg/kg or 15.0% TRR were released during the sequential solubilisation procedure.

In total, 22.4% of the TRR were identified or characterised from the ERR and additional 15.0% of the TRR were characterised by solubilisation from the RRR.

365 DAT: HPLC analysis of the methanol extract of spring wheat straw using method LC14 resulted in a pattern of two peaks (0.011 mg/kg or 21.5% TRR), which were identified as polar fraction. In addition, 0.004 mg/kg or 8.6% TRR were characterised by their extractability with water. Analysis of the same sample using HPLC method LC26 confirmed the polar fraction.

In total, 21.5% of the TRR were identified as polar fraction (carbohydrates), and additional 8.6% of the TRR were characterised by their extractability with water.

Spring wheat grain

30 DAT: Analysis of the concentrated methanol extract of spring wheat grain using HPLC method LC14 yielded a pattern of two peaks, of which the polar fraction (0.019 mg/kg or 2.6% TRR) was identified as the main component. The remaining constituents were characterised by its chromatographic properties (0.003 mg/kg or 0.4% TRR). HPLC analysis of the same sample using method LC26 confirmed the polar fraction. For further characterisation of the polar fraction, the methanol extract was additionally analysed using HPLC method LC21. The analysis resulted in a pattern of six peaks, of which the main component was assigned to fructose / glucose / sucrose (0.012 mg/kg or 1.7% TRR). Thus, the polar fraction was composed of a significant amount of carbohydrates. The presence of carbohydrates was confirmed using HPLC method LC29 with a monosaccharide column.
Analysis of the concentrated water extract using HPLC method LC14 showed one peak, which was identified as polar fraction (0.034 mg/kg or 4.7% TRR) containing significant portions of carbohydrates as main components. The polar peak was confirmed in the same extract using HPLC method LC26.

In order to investigate the residual radioactive residues (RRR), the residues remaining after extraction of spring wheat grain with methanol and water (0.634 mg/kg or 89.2% TRR) were subjected to a sequential solubilisation procedure. Together, 0.511 mg/kg or 71.9% TRR were released during the sequential solubilisation procedure.

In total, 7.8% of the TRR were identified or characterised from the ERR and additional 71.9% of the TRR were characterised by solubilisation from the RRR.

120 DAT: In spring wheat grain, in sum 0.023 mg/kg or 8.5% TRR were characterised by their extractability with methanol and water. Solubilisation after solvent extraction released additional 0.190 mg/kg or 71.1% TRR.

365 DAT: In spring wheat grain, in sum 0.006 mg/kg or 14.0% TRR were characterised by their extractability with methanol and water.

Table 6.6/10 Total identified, characterised and final radioactive residues in spring wheat after treatment with ^{14}C-BAS 351 H

Metabolite / Fraction	Spring Wheat Forage		Spring Wheat Hay	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Plant back interval: 30 DAT				
Total radioactive residue (TRR)	0.270	100.0	1.591	100.0
Extractable radioactive residue (ERR)	0.077	28.4	0.393	24.7
Total identified from ERR	0.053	19.8	0.342	21.5
Total characterised from ERR	0.015	5.7	0.045	2.8
Total identified or characterised from ERR [1)]	**0.069**	**25.5**	**0.387**	**24.3**
Residual radioactive residue (after solvent extraction, RRR)	0.193	71.6	1.198	75.3
Total Identified Radioactive Residues Solubilised from RRR	0.078	28.9	0.283	17.8
Total Characterised Radioactive Residues Solubilised from RRR	0.056	20.9	0.502	31.6
Total identified or characterised radioactive residues solubilised from RRR [2)]	**0.134**	**49.8**	**0.785**	**49.3**
Total identified	**0.131**	**48.7**	**0.625**	**39.3**
Total characterised	**0.072**	**26.6**	**0.547**	**34.4**
Total identified or characterised	**0.203**	**75.3**	**1.171**	**73.6**
Final residue[2)]	**0.011**	**4.2**	**0.068**	**4.3**
Grand Total	**0.214**	**79.5**	**1.239**	**77.9**
Plant back interval: 120 DAT				
Total radioactive residue (TRR)	0.029	100.0	0.146	100.0
Extractable radioactive residue (ERR)	0.008	27.5	0.033	22.7
Total identified from ERR	0.007	23.8	0.022	15.0
Total characterised from ERR	0.001	3.7	0.011	7.7
Total identified or characterised from ERR [1)]	**0.008**	**27.5**	**0.033**	**22.7**
Residual radioactive residue (after solvent extraction, RRR)	0.021	72.5	0.113	77.3
Total Characterised Radioactive Residues Solubilised from RRR	0.009	30.4	0.035	23.9
Total characterised radioactive residues solubilised from RRR [2)]	**0.009**	**30.4**	**0.035**	**23.9**
Total identified	**0.007**	**23.8**	**0.022**	**15.0**
Total characterised	**0.010**	**34.1**	**0.046**	**31.6**
Total identified or characterised	**0.017**	**57.9**	**0.068**	**46.6**
Final residue	**0.008**	**25.9**	**0.064**	**44.1**
Grand Total	**0.025**	**83.8**	**0.133**	**90.7**
Plant back interval: 365 DAT				
Total radioactive residue (TRR)	**-**	**-**	0.070	100.0
Total identified from ERR	**-**	**-**	0.013	19.0
Total characterised from ERR	**-**	**-**	0.004	6.2
Total identified or characterised from ERR [1)]	**-**	**-**	**0.018**	**25.2**
Residual radioactive residue (after solvent extraction, RRR)	**-**	**-**	0.052	74.8
Sum of RRR and Total identified or characterised from ERR	**-**	**-**	**0.070**	**100.0**

[1)] ERR = Extractable Radioactive Residue (methanol extract and water extract)
[2)] see Table 6.6/7

Table 6.6/11 Total identified, characterised and final radioactive residues in spring wheat after treatment with ^{14}C-BAS 351 H

Metabolite / Fraction	Spring Wheat Straw		Spring Wheat Grain	
	[mg/kg]	[%TRR]	[mg/kg]	[%TRR]
Plant back interval: 30 DAT				
Total radioactive residue (TRR)	1.107	100.0	0.711	100.0
Extractable radioactive residue (ERR)	0.262	23.7	0.077	10.8
Total identified from ERR	0.185	16.8	0.052	7.4
Total characterised from ERR	0.066	5.9	0.003	0.4
Total identified or characterised from ERR [1)]	**0.251**	**22.7**	**0.055**	**7.8**
Residual radioactive residue (after solvent extraction, RRR)	0.845	76.3	0.634	89.2
Total Identified Radioactive Residues Solubilised from RRR	0.147	13.2	0.485	68.2
Total Characterised Radioactive Residues Solubilised from RRR	0.498	45.0	0.026	3.7
Total identified or characterised radioactive residues solubilised from RRR [2)]	**0.645**	**58.3**	**0.511**	**71. 9**
Total identified	**0.332**	**30.0**	**0.537**	**75.6**
Total characterised	**0.564**	**51.0**	**0.029**	**4.1**
Total identified or characterised	**0.896**	**81.0**	**0.567**	**79.7**
Final residue	**0.035**	**3.1**	**0.041**	**5.7**
Grand Total	**0.931**	**84.1**	**0.608**	**85.5**
Plant back interval: 120 DAT				
Total radioactive residue (TRR)	0.127	100.0	0.267	100.0
Extractable radioactive residue (ERR)	0.028	22.4	0.023	8.5
Total identified from ERR	0.020	15.6		
Total characterised from ERR	0.009	6.8	0.023	8.5
Total identified or characterised from ERR [1)]	**0.028**	**22.4**		
Residual radioactive residue (after solvent extraction, RRR)	0.099	77.6	0.244	91.5
Total characterised radioactive residues solubilised from RRR	0.019	15.0		
Total characterised radioactive residues solubilised from RRR [2)]	**0.019**	**15.0**	**0.190**	**71.1**
Total identified	**0.020**	**15.6**		
Total characterised	**0.028**	**21.8**	**0.212**	**79.6**
Total identified or characterised	**0.047**	**37.4**		
Final residue	**0.071**	**55.8**	**0.021**	**7.9**
Grand Total	**0.118**	**93.1**	**0.233**	**87.5**
Plant back interval: 365 DAT				
Total radioactive residue (TRR)	0.049	100.0	0.041	100.0
Total identified from ERR	0.011	21.5	-	-
Total characterised from ERR	0.004	8.6	0.006	14.0
Total identified or characterised from ERR [1)]	**0.015**	**30.0**	-	-
Residual radioactive residue (after solvent extraction, RRR)	0.034	70.0	-	-
Sum of RRR and Total identified or characterised from ERR	**0.049**	**100.0**	-	-

[1)] ERR = Extractable Radioactive Residue (methanol extract and water extract)
[2)] see Table 6.6/7

4. Proposed metabolic pathway

The radioactive residues in the extracts and solubilisates of all rotational crop matrices mainly consisted of a highly polar fraction which was identified to contain primarily carbohydrates (fructose, glucose and sucrose and further components of similar polarity). The unchanged parent compound BAS 351 H was only found as minor component in samples of immature (30 DAT) and mature lettuce (30 and 120 DAT, ≤ 2.5% TRR, ≤ 0.001 mg/kg). Additional minor medium polar degradation products were characterised by their chromatographic properties (0.3 - 4.2% TRR, each below 0.04 mg/kg and one peak representing 0.0013 mg/kg or 10.2% TRR).
Since rapid decomposition of BAS 351 H and formation of metabolites in soil occurred, the composition of the residues in the analysed rotational crop matrices implies the decomposition of the parent compound and formation of degradation products in soil and after plant uptake. The incorporation of radiolabelled carbon atoms from the aromatic ring system of bentazone into sugar molecules requires the transformation of the active substance or its degradation products / soil metabolites into suitable compounds for the biosynthesis of carbohydrates. Therefore, it is likely that C1 and / or C2 entities derived from bentazone enter the sugar anabolic pathway. In consequence, radiolabelled sugar moieties can also be incorporated into polysaccharides such as cell wall polymers or starch (see Figure 6.6/1).

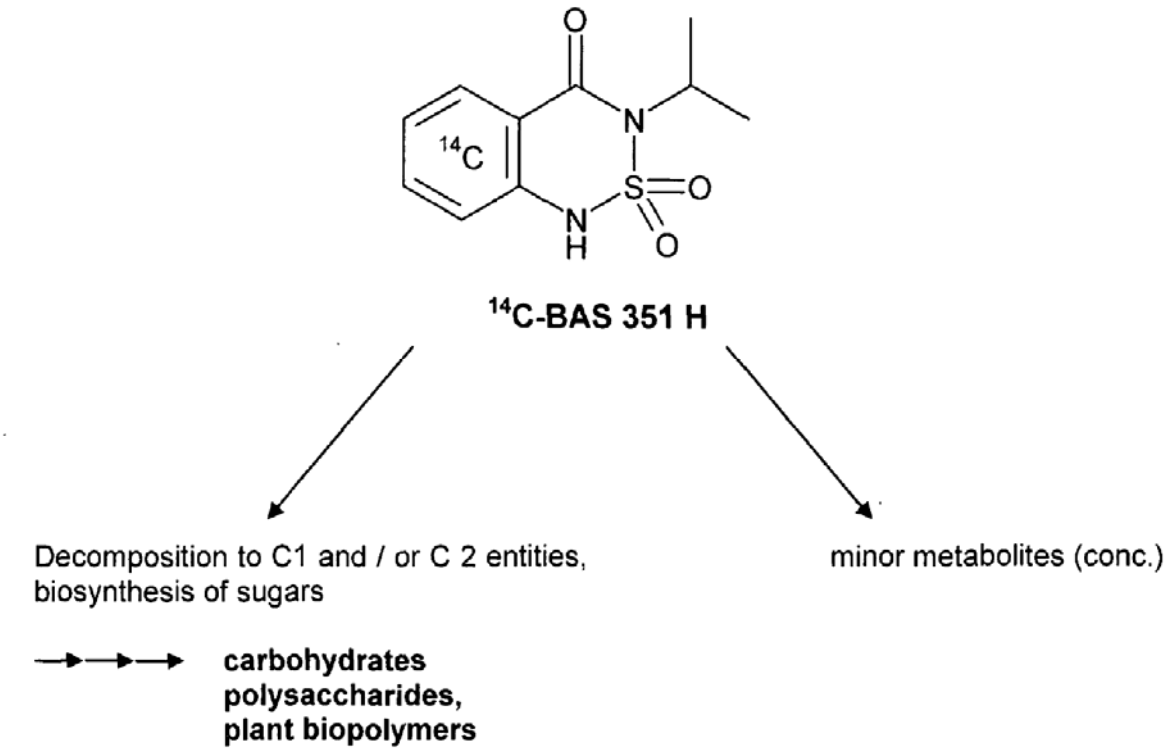
5. Storage stability of residues

Storage stability investigations were performed in four representative matrices of the plant back interval of 30 DAT at the beginning and at the end of the study.
All samples were stored in a freezer at approximately -18 °C or below during the course of the study. A comparison of the extractabilities and of the metabolite patterns (HPLC analyses) obtained at the beginning and at the end of the investigation period (representative samples of mature lettuce, spring wheat straw and grain, all 30 DAT) showed that there was no relevant change in the nature of the radioactive residues of BAS 351 H during storage of the plant samples over the period of investigation. The stability in stored extracts was demonstrated for representative samples of spring wheat forage, straw and grain over periods of 11 to 19 months.

III. CONCLUSION

The metabolism of bentazone in succeeding crops was investigated in wheat, radish and lettuce cultivated at three different replant intervals for all crops (30, 120 and 365 DAT).
Significant translocation of radioactive residues from soil into the plants was observed for the plant back interval of 30 DAT which declined rapidly for longer aging periods of 120 and 365 days. The residue concentration in the top soil layer after aging and ploughing decreased slightly with increasing plant back intervals.
The total radioactive residues (TRR) in lettuce (immature and mature samples) did not exceed 0.128 mg/kg for all plant back intervals. The TRR in white radish top was 0.168 mg/kg at a plant back interval of 30 DAT, decreasing to 0.019 mg/kg after 120 DAT and to 0.003 mg/kg (TRR combusted) after 365 DAT. The total radioactive residues in roots of mature crop decreased from 0.128 mg/kg (30 DAT), to 0.012 mg/kg (120 DAT) and finally to 0.001 mg/kg (365 DAT, TRR combusted). In spring wheat, the highest residue levels were measured in hay (ranging from 0.070 to 1.591 mg/kg, for 30 DAT and 365 DAT, respectively) and straw (0.049 to 1.107 mg/kg, for 30 DAT and 365 DAT, respectively). The total radioactive residues in grain accounted for 0.041 to 0.711 mg/kg.
Analysis of the extracts and solubilisates after solvent extraction using the HPLC resulted for all crops, matrices and DAT intervals in an early eluting peak or peak group. This peak or peak group represented the main component in all matrices and for all aging intervals and was identified as polar fraction. The composition of the polar fraction was further investigated using an HPLC method suitable for saccharide analysis. The main portion of the residues of the polar fraction corresponded to glucose, fructose and sucrose (>50% ROI), showing composition of the polar fraction of carbohydrates.
Bentazone (BAS 351 H) and/or its soil metabolites were taken up and transformed in the rotational crops primarily into sugars (glucose, fructose and sucrose and further components of similar polarity) which were without exception the most abundant components in all matrices examined (methanol / water extracts and solubilisates after solvent extraction). The unchanged parent molecule BAS 351 H was found as minor component in samples of immature (30 DAT) and mature lettuce (30 and 120 DAT) in concentrations of <0.0013 mg/kg and 1.2% TRR only. Additional medium polar degradation products were detected in minor concentrations.
The composition of the residues in the analysed rotational crop matrices implies the decomposition of the parent compound in soil and after plant uptake. The incorporation of radiolabelled carbon atoms from the aromatic ring system of bentazone into sugar molecules requires the transformation of the active substance or its degradation products / soil metabolites into suitable compounds for the biosynthesis of carbohydrates. Therefore, it is likely that C1 and / or C2 entities of bentazone enter the sugar anabolic pathway. As a consequence radiolabelled sugar moieties can also be found in biopolymers such as cell wall polymers or starch.

Figure 6.6/1 Metabolic pathway of ^{14}C-BAS 351 H in rotational crops



6.6.3 Field trials on representative crops

Even though confined rotational crop studies with bentazone showed no relevant metabolites, three field studies were conducted to show rotational crops following a soybean culture. No uptake of residues occurred when various crops (radish, lettuce, snap bean, spinach, sorghum, wheat, turnip, mustard, corn, alfalfa and sugar beet) were grown as emergency replants or fall and annual rotational crops following typical soybean culture, which was treated twice with the bentazone formulation BASAGRAN at a rate of 1.12 kg a.s./ha.

Further information on the field rotational crop studies is presented in the Full Report (1998) on the active substance bentazone.

6.7 Proposed residue definition and maximum residue levels

6.7.1 Proposed residue definition

6.7.1.1 Plant matrices

In Regulation (EC) No 396/2005 as amended by Regulation (EU) No 893/2010, the following residue definition for both risk assessment and monitoring has been established for products of plant origin:

Bentazone (sum of bentazone and the conjugates of 6-OH-bentazone and 8-OH-bentazone expressed as bentazone)

The same residue definition was concluded in the 1998 evaluation of bentazone by the JMPR.

6.7.1.2 Animal matrices

In Regulation (EC) No 396/2005 as amended by Regulation (EU) No 893/2010, the following residue definition for both risk assessment and monitoring has been established for products of animal origin:

Bentazone

The JMPR evaluation 1998 also defined bentazone as major component of the residues in animal matrices.

For the reason of bentazone, conjugated 6-OH- and 8-OH-bentazone being the main residues in most feed items it is proposed to establish the residue definition for animal matrices as originally proposed in the Full Report as:

Bentazone (sum of bentazone and the conjugates of 6-OH-bentazone and 8-OH-bentazone expressed as bentazone)

6.7.2 Proposed maximum residue levels (MRLs) and justification

MRLs were proposed based on the rounded MRLs derived with the OECD calculator (OECD calculator spreadsheet: http://www.oecd.org/document/34/0,3746,en_2649_37465_48447010_1_1_1_37465,00.html).

Established / existing / current MRLs refer to MRLs under Regulation (EC) No 396/2005 as amended by Regulation (EU) No 893/2010.

The residue data for bentazone in the present dossier was analysed mainly using two different analytical methods (BASF method no 438/1 and 438/2, exceptions are minor use residue data for valerian root and wooly foxglove). BASF methods 438/1 and 438/2 analyse for parent compound bentazone and the conjugates of 6-OH-bentazone and 8-OH-bentazone with an LOQ of 0.02 mg/kg and 0.01 mg/kg for each analyte, respectively. Thus, the LOQ for the sum of bentazone and its metabolites is 0.06 mg/kg and 0.03 mg/kg for the two different methods, respectively. Therefore an overall LOQ of 0.06 mg/kg is considered suitable for all plant commodities while for animal matrices the LOQ of 0.03 mg/kg should apply.

Therefore it is proposed to:

1. Reduce the **default MRL** for all **plant commodities** in accordance with new MRL classes from 0.1 mg/kg to **0.06 mg/kg**
2. MRL calculations should be done using LOQs of 0.06 mg/kg and 0.03 mg/kg or a combination of both, whichever is available
3. The MRL of 0.1 mg/kg should be kept for all commodities where uses are intended and no residues were observed
4. A **default MRL of 0.03 mg/kg** should apply for all **animal food items**, according to the proposed residue definition and LOQ of the analytical method

Calculations of all MRLs presented in this dossier are based on the lowest available data.

6.7.2.1 Root and tuber vegetables

Potato

In 8 trials conducted in 2009 in Southern Europe, the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at harvest (PHI F) after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha:

-	Northern Europe
<0.03 (4x), 0.03, 0.04 (2x), 0.08	Southern Europe

In the Annex II Dossier (1995), 13 trials (Northern Europe) on potatoes were presented with total residues at or below the LOQ of 0.06 mg/kg at 28-140 DALA (see below). The application rate was 1 x 0.96 or 1.2 kg a.s./ha.

<0.06 (12), 0.06	Northern Europe - AII Dossier 1995
-	Southern Europe - AII Dossier 1995

MRL calculation was performed using all residue values presented above [see Table 6.7/1]. In case of residues below the limit of quantitation of the analytical method applied, 0.03 mg/kg and 0.06 mg/kg were used for calculation purposes, respectively.

Table 6.7/1 MRL calculation for potato

OECD Calculator	Total Bentazone [mg/kg]	
	N-EU	S-EU
Highest residue	0.06	0.08
Mean + 4 SD	0.06	0.11
CF x 3 Mean	0.07	0.09
Rounded MRL	0.07	**0.15**
STMR	0.06	0.03

According to Appendix D (Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs, 7525/VI/95, rev. 8, 01.02.2008), it is allowed to extrapolate from potatoes to tropical root vegetables.

An EU MRL of 0.15 mg/kg was calculated for potatoes. Therefore, it is proposed to raise the established EU MRL of 0.1 mg/kg to **0.15 mg/kg** for **potatoes and tropical root vegetables**.

6.7.2.2 Bulb vegetables

Bulb onion

In 12 trials conducted in 1999-2009 in Northern (4 trials) and Southern Europe (8 trials), the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at harvest (PHI F):

<0.06 (4x)	Northern Europe
<0.03 (7x), 0.04	Southern Europe

Since all residues were below the LOQ in Northern Europe, four residue trials are sufficient for this region. Furthermore, those trials were performed with an exaggerated application rate of 1.55 kg a.s./ha compared to the current registered cGAP with an application rate of 0.96 kg a.s./ha. MRL calculation was performed [see Table 6.7/2]. In case of residues below the limit of quantitation of the analytical method applied, 0.03 mg/kg and 0.06 mg/kg, respectively, were used for calculation purposes.

Table 6.7/2 MRL calculation for bulb onion

OECD Calculator	Total Bentazone [mg/kg]	
	N-EU	S-EU
Highest residue	0.06	0.04
Mean + 4 SD	0.06	0.05
CF x 3 Mean	0.06	0.04
Rounded MRL	**0.06**	0.05
STMR	0.06	0.03

According to Appendix D (Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs, 7525/VI/95, rev. 8, 01.02.2008), it is allowed to extrapolate from bulb onions to garlic and shallots.

An EU MRL of 0.06 mg/kg was calculated for bulb onions. This value is covered by the existing EU MRL and it is thus proposed to maintain the established EU MRLs of **0.1 mg/kg** for **onions, garlic and shallots**.

Spring onion

In 4 trials conducted in 2009 in Northern (2 trials) and Southern Europe (2 trials), the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at harvest (PHI F) after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha:

<0.03, 0.06	Northern Europe
<0.03 (2x)	Southern Europe

MRL calculation was performed [see Table 6.7/3]. In case of residues below the limit of quantitation of the analytical method applied, 0.03 mg/kg were used for calculation purposes.

Table 6.7/3 MRL calculation for spring onion

OECD Calculator	Total Bentazone [mg/kg]
	N-EU + S-EU
Highest residue	0.06
Mean + 4 SD	0.098
CF x 3 Mean	0.056
Rounded MRL	**0.1**
STMR	0.03

N/A not applicable

According to Appendix D (Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs, 7525/VI/95, rev. 9, 24.03.2011), it is allowed to extrapolate from spring onions to Welsh onions and chives as well as to leek.

AN EU MRL of 0.1 mg/kg was calculated for spring onion. Therefore, it is proposed to maintain the established EU MRLs of **0.1 mg/kg** for **spring onions, Welsh onions and chives and for leek**.

6.7.2.3 Fruiting vegetables

Sweet corn

In 2 trials conducted in 2007 in Northern (1 trial) and Southern Europe (1 trial), the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at PHI 28±1 days after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha:

<0.03	Northern Europe
0.06	Southern Europe

According to Appendix D (Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs, 7525/VI/95, rev. 8, 01.02.2008), it is allowed to extrapolate from immature maize to sweet corn. The following residues were found in immature maize cobs without husks at PHI 28±1 days after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha:

<0.03 (2x), 0.03, 0.10, 0.20 (cobs w/o husks)	Northern Europe
<0.03 (2x), 0.04, 0.05, 0.10 (cobs w/o husks)	Southern Europe

MRL calculation was performed considering residue values for both sweet corn and immature maize. In case of residues below the limit of quantitation of the analytical method applied, 0.03 mg/kg and 0.06 mg/kg, respectively, were used for calculation purposes.

Table 6.7/4 MRL calculation for sweet corn

OECD Calculator	Total Bentazone [mg/kg]	
	N-EU	S-EU
Highest residue	0.20	0.10
Mean + 4 SD	0.35	0.16
CF x 3 Mean	0.14	0.12
Rounded MRL	**0.4**	0.2
STMR	0.03	0.045

An EU MRL of 0.4 mg/kg was calculated for **sweet corn**. This MRL is higher than the one proposed in the recent EFSA reasoned opinion (EFSA Journal 2010; 8(5):1617) which is due to the use of the OECD MRL calculator. Therefore, it is proposed to raise the established EU MRL of 0.3 mg/kg to **0.4 mg/kg**.

6.7.2.4 Fresh herbs

Thyme

In 4 trials conducted in 2005-2006 in Southern Europe, the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at harvest (PHI F) after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha:

-	Northern Europe
<0.05, 0.061, 0.072, 0.087	Southern Europe

Since thyme is an herb predominantly grown in the Mediterranean area, the Southern European data is considered appropriate for MRL calculation.
MRL calculation was performed [see Table 6.7/5]. In case of residues below the limit of quantitation of the analytical method applied, 0.05 mg/kg were used for calculation purposes.

Table 6.7/5 MRL calculation for thyme

OECD Calculator	Total Bentazone [mg/kg]	
	N-EU	S-EU
Highest residue	N/A	0.087
Mean + 4 SD	N/A	0.13
CF x 3 Mean	N/A	0.17
Rounded MRL	N/A	**0.2**
STMR	N/A	0.067

N/A not applicable

An EU MRL of 0.2 mg/kg was calculated. However, it is proposed to maintain the EU MRL of **10 mg/kg** for **thyme** as recommended by EFSA in the Reasoned Opinion on the modification of the existing MRLs for bentazone in legume vegetables and fresh herbs (EFSA Journal 2011;9(5):2188).

6.7.2.5 Legume vegetables

Beans and peas with pods

According to Appendix D (Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs, 7525/VI/95, rev. 8, 01.02.2008), it is allowed to extrapolate from beans with pods to peas with pods.

In 16 trials on beans conducted in 2008-2009 in Northern (7 trials) and Southern Europe (9 trials), the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at PHI 28±1 days or later if higher residues occurred after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha:

0.03, 0.04, 0.05, 0.06, 0.07, 0.09, 0.10	Northern Europe
<0.03 (3x), 0.03, 0.04, 0.06, 0.08, 0.11, 0.21	Southern Europe

In 2 trials on peas conducted in 2007 in Northern Europe, the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at PHI 28±1 days after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha:

0.04, 0.05	Northern Europe
-	Southern Europe

MRL calculation was performed using all residue values presented above [see Table 6.7/6]. In case of residues below the limit of quantitation of the analytical method applied, 0.03 mg/kg were used for calculation purposes.

Table 6.7/6 MRL calculation for beans and peas with pods

OECD Calculator	Total Bentazone [mg/kg]	
	N-EU	S-EU
Highest residue	0.10	0.21
Mean + 4 SD	0.15	0.31
CF x 3 Mean	0.18	0.16
Rounded MRL	0.2	**0.3**
STMR	0.06	0.04

An MRL of 0.3 mg/kg was calculated for beans with pods. Therefore, it is proposed to adjust the established EU MRL of 0.1 mg/kg to **0.3 mg/kg** for **beans with pods** as recommended by EFSA in the Reasoned Opinion on the modification of the existing MRLs for bentazone in legume vegetables and fresh herbs (EFSA Journal 2011;9(5):2188).
For peas with pods, it is proposed to maintain the established EU MRL of **0.5 mg/kg** for **peas with pods** as recommended by EFSA in the Reasoned Opinion on the modification of the existing MRLs for bentazone in legume vegetables and fresh herbs (EFSA Journal 2011;9(5):2188).

Beans and peas without pods

According to Appendix D (Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs, 7525/VI/95, rev. 8, 01.02.2008), no extrapolation to peas without pods is pointed out. However, in the EFSA Reasoned Opinion (EFSA Journal 2011;9(5):2188), it was extrapolated from beans without pods.

In 15 trials on beans conducted in 2008-2009 in Northern (6 trials) and Southern Europe (9 trials), the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at PHI 28±1 days or later if higher residues occurred after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha:

<0.03 (5x), 0.03	Northern Europe
<0.03 (8x), 0.04	Southern Europe

In 2 trials on peas conducted in 2007 in Northern Europe, the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at PHI 28±1 days or later if higher residues occurred after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha:

<0.03 (2x)	Northern Europe
-	Southern Europe

MRL calculation was performed using all residue values presented above [see Table 6.7/7]. In case of residues below the limit of quantitation of the analytical method applied, 0.03 mg/kg were used for calculation purposes.

Table 6.7/7 MRL calculation for beans and peas without pods

OECD Calculator	Total Bentazone [mg/kg]	
	N-EU	S-EU
Highest residue	0.03	0.04
Mean + 4 SD	0.03	0.04
CF x 3 Mean	0.04	0.04
Rounded MRL	0.04	**0.05**
STMR	0.03	0.03

An EU MRL of 0.05 mg/kg was calculated for beans without pods and recommended by EFSA in the Reasoned Opinion on the modification of the existing MRLs for bentazone in legume vegetables and fresh herbs (EFSA Journal 2011;9(5):2188). Despite this, an MRL of **0.06 mg/kg** is proposed for **beans without pods** since it is not reasonable to set an MRL below the default MRL, which is proposed as 0.06 mg/kg in this document.
For peas without pods, it is proposed to maintain the established EU MRL of **0.2 mg/kg** for **peas without pods** as recommended by EFSA in the Reasoned Opinion on the modification of the existing MRLs for bentazone in legume vegetables and fresh herbs (EFSA Journal 2011;9(5):2188).

6.7.2.6 Pulses

Dried beans

In 15 trials conducted in 1999-2010 in Northern (5 trials) and Southern Europe (10 trials), the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at harvest (PHI F):

<0.06 (4x), 0.06	Northern Europe
<0.03 (2x), <0.06 (7x), 0.06	Southern Europe

Overdosed trials (1.536-1.67 kg a.s./ha) in Northern (5 trials) and Southern Europe (8 trials) compared to the current registered cGAP with an application rate of 0.96 kg a.s./ha were included as total bentazone residues were at or below the LOQ of 0.06 mg/kg. The trials performed with 0.96 kg a.s./ha however were not considered for MRL determination since the application was performed rather close to harvest and therefore the resulting residues are comparatively high.
MRL calculation was performed using the residue values presented above [see Table 6.7/8]. In case of residues below the limit of quantitation of the analytical method applied, 0.03 mg/kg and 0.06 mg/kg, respectively, were used for calculation purposes.

Table 6.7/8 MRL calculation for dried beans

OECD Calculator	Total Bentazone [mg/kg]	
	N-EU	S-EU
Highest residue	0.06	0.06
Mean + 4 SD	0.06	0.105
CF x 3 Mean	0.84	0.065
Rounded MRL	0.09	**0.1**
STMR	0.06	0.06

According to Appendix D (Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs, 7525/VI/95, rev. 8, 01.02.2008), it is allowed to extrapolate from green beans with pods to the whole group of pulses (dry) if the application of the active substance is performed before the consumable part of the crop has started to form.

An EU MRL of 0.1 mg/kg was calculated for dried beans, while for green beans with pods, an MRL of **0.3 mg/kg** has been recommended by EFSA in the Reasoned Opinion on the modification of the existing MRLs for bentazone in legume vegetables and fresh herbs (EFSA Journal 2011;9(5):2188) (see above). Therefore, it is proposed to adjust the established EU MRL of 0.1 mg/kg for the **whole group of pulses (dry)** to 0.3 mg/kg.

Dried peas

The trials performed with 0.96 kg a.s./ha in dried peas were not considered for MRL determination since the application was performed rather close to harvest and therefore the resulting residues are quite high.

According to Appendix D (Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs, 7525/VI/95, rev. 8, 01.02.2008), it is allowed to extrapolate from green beans with pods to the whole group of pulses (dry) if the application of the active substance is performed before the consumable part of the crop has started to form. Therefore, it is proposed to adjust the established EU MRL of 0.1 mg/kg for the whole group of pulses (dry) to 0.3 mg/kg (see above).

6.7.2.7 Oilseeds

Soybean

In 15 trials conducted in 2007-2009 in Northern (3 trials) and Southern Europe (12 trials), the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at harvest (PHI F) after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha:

<0.03 (3x)	Northern Europe
<0.03 (12x)	Southern Europe

Since all residues from Northern European trials were below the LOQ and soybean is considered to be a minor crop for the Northern region (according to Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs, 7525/VI/95, rev. 8, 01.02.2008), the number of residue trials is sufficient.
MRL calculation was performed [see Table 6.7/9]. In case of residues below the limit of quantitation of the analytical method applied, 0.03 mg/kg were used for calculation purposes.

Table 6.7/9 MRL calculation for soybean

OECD Calculator	Total Bentazone [mg/kg]	
	N-EU	S-EU
Highest residue	0.03	0.03
Mean + 4 SD	0.03	0.03
CF x 3 Mean	0.03	0.03
Rounded MRL	**0.03**	**0.03**
STMR	0.03	0.03

An EU MRL of 0.03 mg/kg was determined for soybean.

Linseed

In 4 trials conducted in 1999 in Southern Europe, the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at harvest (PHI F):

-	Northern Europe
<0.06 (4x)	Southern Europe

Even though all trials were overdosed (about 1.5 kg a.s./ha), compared to the current registered cGAP with an application rate of 0.96 kg a.s./ha, residues were below the LOQ. If all residues are below the LOQ, two residue trials per region are sufficient. Therefore, the four trials performed in Southern Europe are assumed to cover the Northern European region as well.
MRL calculation was performed [see Table 6.7/10]. In case of residues below the limit of quantitation of the analytical method applied, 0.06 mg/kg were used for calculation purposes.

Table 6.7/10 MRL calculation for linseed

OECD Calculator	Total Bentazone [mg/kg]	
	N-EU	S-EU
Highest residue	N/A	0.06
Mean + 4 SD	N/A	0.06
CF x 3 Mean	N/A	0.06
Rounded MRL	N/A	**0.06**
STMR	N/A	0.06

N/A not applicable

An EU MRL of 0.06 mg/kg was determined for linseed.

According to Appendix D (Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs, 7525/VI/95, rev. 8, 01.02.2008), it is allowed to extrapolate from soybean to the whole group of oilseeds except peanuts. To cover the calculated MRL values of both soybean and linseed, it is proposed to maintain the established EU MRL of **0.1 mg/kg** for the **whole group of oilseeds except peanuts**.

6.7.2.8 Cereals

Maize

In 28 trials conducted in 1998-2010 in Northern (12 trials) and Southern Europe (16 trials), the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found in maize grain at harvest (PHI F):

<0.03 (6x), 0.04 (2x), <0.06 (4x)	Northern Europe
<0.03 (8x), <0.06 (8x)	Southern Europe

Results from exaggerated trials (1 x 1.55 kg a.s./ha compared to the current registered cGAP with an application rate of 0.96 kg a.s./ha) are also included (2 N-EU, 8 S-EU); the residues were below the LOQ of 0.06 mg/kg.
MRL calculation was performed [see Table 6.7/11]. In case of residues below the limit of quantitation of the analytical method applied, 0.03 mg/kg and 0.06 mg/kg were used for calculation purposes, respectively.

Table 6.7/11 MRL calculation for maize grain

OECD Calculator	Total Bentazone [mg/kg]	
	N-EU	S-EU
Highest residue	0.06	0.06
Mean + 4 SD	0.10	0.11
CF x 3 Mean	0.06	0.05
Rounded MRL	**0.1**	0.06
STMR	0.035	0.045

According to Appendix D (Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs, 7525/VI/95, rev. 8, 01.02.2008), it is allowed to extrapolate from maize to millet and sorghum.

An EU MRL of 0.1 mg/kg was calculated for maize. Therefore, it is proposed to maintain the established EU MRLs of **0.1 mg/kg** for **maize, millet and sorghum**.

Wheat and barley

According to Appendix D (Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs, 7525/VI/95, rev. 8, 01.02.2008), it is allowed to extrapolate from barley, oats, rye, triticale or wheat to the remaining four crops. Therefore, wheat and barley residue trials are combined to determine a group MRL for cereals.

In 5 trials on barley conducted in 1999 in Southern Europe, the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at harvest (PHI F):

-	Northern Europe
<0.06 (5x)	Southern Europe

Results from exaggerated trials (1 x 1.55 kg a.s./ha compared to the current registered cGAP with an application rate of 0.96 kg a.s./ha) were used.

In 11 trials on wheat conducted in 1998 and 2010 in Northern (8 trials) and Southern Europe (3 trials), the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at harvest (PHI F):

<0.03 (8x)	Northern Europe
<0.06 (3x)	Southern Europe

Results from exaggerated trials (1 x 1.55 kg a.s./ha compared to the current registered cGAP with an application rate of 0.96 kg a.s./ha) were used in Southern Europe.

MRL calculation was performed using all residue values presented above [see Table 6.7/12]. In case of residues below the limit of quantitation of the analytical method applied, 0.03 mg/kg and 0.06 mg/kg were used for calculation purposes, respectively.

Table 6.7/12 MRL calculation for cereal grain

OECD Calculator	Total Bentazone [mg/kg]	
	N-EU	S-EU
Highest residue	0.03	0.06
Mean + 4 SD	0.03	0.06
CF x 3 Mean	0.03	0.06
Rounded MRL	0.03	**0.06**
STMR	0.03	0.06

An EU MRL of 0.06 mg/kg was determined for wheat and barley. However, it is proposed to maintain the established EU MRL of **0.1 mg/kg** for **barley, oats, rye, triticale and wheat**.

Maize forage

Total Bentazone
Maize forage is a feedstuff. In 28 trials conducted in 1998-2010 in Northern (12 trials) and Southern Europe (16 trials), the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at harvest in rest of plants:

<0.03, <0.06 (4x), 0.49, 0.74, 0.77, 1.11, 1.3, 1.61, 1.78	Northern Europe
<0.06 (7x), 0.07 (2x), 0.12, 0.14, 0.26, 0.39, 0.57, 0.69, 1.29	Southern Europe

Results from exaggerated trials (1 x 1.55 kg a.s./ha compared to the current registered cGAP with an application rate of 0.96 kg a.s./ha) are also included (2 N-EU, 8 S-EU); the residues were below the LOQ of 0.06 mg/kg in Northern Europe and <0.06-0.07 mg/kg in Southern Europe.

The resulting STMR and HR values used for feed burden calculations are given below.

	N-EU	**S-EU**
STMR	0.74	0.07
HR	1.78	1.29

The HR of maize forage (sum of bentazone and the conjugates of 6-OH-bentazone and 8-OH-bentazone expressed as bentazone) is the main contributor to the feed burden of cattle. For exact calculation of the respective feed burden of parent bentazone and each of the two metabolites, residues of the respective single residue were used. Basis for the calculations are the trials listed above.

Bentazone
In 28 trials conducted in 1998-2010 in Northern (12 trials) and Southern Europe (16 trials), the following residues of the parent compound bentazone (in mg/kg) were found at harvest in rest of plants:

<0.01 (4x), 0.01, <0.02 (4x), 0.02, 0.03, 0.04	Northern Europe
<0.01 (4x), <0.02 (8x), 0.02 (3x), 0.13	Southern Europe

The resulting STMR and HR values of bentazone used for feed burden calculations are given below.

	N-EU	**S-EU**
STMR	0.02	0.02
HR	0.04	0.13

6-OH-Bentazone
In 28 trials conducted in 1998-2010 in Northern (12 trials) and Southern Europe (16 trials), the following residues of 6-OH-bentazone (in mg/kg) were found at harvest in rest of plants:

<0.01, <0.02 (4x), 0.47, 0.72, 0.74, 1.09, 1.20, 1.61, 1.79	Northern Europe
<0.02 (7x), 0.03, 0.05, 0.10, 0.11, 0.24, 0.37, 0.57, 0.58, 1.26	Southern Europe

The resulting STMR and HR values of 6-OH-bentazone used for feed burden calculations are given below.

	N-EU	S-EU
STMR	0.47	0.03
HR	1.79	1.26

8-OH-Bentazone
In 28 trials conducted in 1998-2010 in Northern (12 trials) and Southern Europe (16 trials), the following residues of 6-OH-bentazone (in mg/kg) were found at harvest in rest of plants:

<0.01 (2x), 0.01, <0.02 (4x), 0.02, 0.03, 0.06, 0.07, 0.08	Northern Europe
< 0.01 (4x), 0.01 (2x), <0.02 (8x), 0.02 (2x)	Southern Europe

The resulting STMR and HR values of 8-OH-bentazone used for feed burden calculations are given below.

	N-EU	S-EU
STMR	0.02	0.02
HR	0.08	0.02

Wheat and barley straw

Wheat and barley straw are feedstuffs. In 5 trials on barley conducted in 1999 in Southern Europe, the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at harvest:

-	Northern Europe
0.10, 0.14, 0.16, 0.38, 0.65	Southern Europe

Results from exaggerated trials (1 x 1.55 kg a.s./ha compared to the current registered cGAP with an application rate of 0.96 kg a.s./ha) were used.

In 11 trials on wheat conducted in 1998 and 2010 in Northern (8 trials) and Southern Europe (3 trials), the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at harvest (PHI F):

<0.03 (6x), 0.03, 0.06	Northern Europe
0.08, 0.09, 0.10	Southern Europe

Results from exaggerated trials (1 x 1.55 kg a.s./ha compared to the current registered cGAP with an application rate of 0.96 kg a.s./ha) were used in Southern Europe.

The resulting STMR and HR values used for feed burden calculations are given below.

	N-EU	S-EU
STMR	0.03	0.12
HR	0.06	0.65

6.7.2.9 Herbal infusions (dried)

Valerian root

In 4 trials conducted in 2001-2003 in Northern Europe, the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at harvest (PHI F) after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha:

<0.13 (4x)	Northern Europe
-	Southern Europe

Since the use in valerian roots is only intended in Northern Europe, the number of trials performed is sufficient for MRL determination. The methods described in ENV/JM/MONO(2011)2 of 01 March 2011 were used for MRL calculation [see Table 6.7/13]. In case of residues below the limit of quantitation of the analytical method applied, 0.13 mg/kg were used for calculation purposes.

Table 6.7/13 MRL calculation for valerian root

OECD Calculator	Total Bentazone [mg/kg]	
	N-EU	S-EU
Highest residue	0.13	N/A
Mean + 4 SD	0.13	N/A
CF x 3 Mean	0.13	N/A
Rounded MRL	**0.15**	N/A
STMR	0.13	N/A

N/A not applicable

According to Appendix D (Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs, 7525/VI/95, rev. 8, 01.02.2008), it is allowed to extrapolate from any single cultivated crop of herbal roots to roots of herbal infusions and spices.

An EU MRL of **0.15 mg/kg** was determined for valerian roots. Therefore, it is proposed to adjust the established EU MRL of 0.1 mg/kg for **roots of herbal infusions and spices** to 0.15 mg/kg.

6.7.2.10 Others

Alfalfa

Alfalfa forage is a feedstuff. In 6 trials conducted in 2007 in Northern (2 trials) and Southern Europe (4 trials), the following total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) were found at harvest (see also chapter 6.10):

(1.419, 5.40)	Northern Europe
0.594, 0.70, 0.709, 0.72	Southern Europe

All trials were underdosed (0.6 kg a.s./ha compared to the current registered cGAP with an application rate of 0.96 kg a.s./ha). Therefore the maximum residue of alfalfa green matter and hay was chosen per trial, taking all sampling times into account (DALA 14-41). Trials from Northern Europe were not taken into account for feed burden calculations, since application took place later than the intended GAP resulting in residues far above trials with early application.
In the Annex II Dossier (1995), 6 trials on alfalfa were presented with total residues ranging from 0.07 to 1.43 mg/kg at DALA 29-262 (see below). The application rate was 2 x 0.96 kg a.s./ha in one trial and 1 x 1.44 kg a.s./ha in the others.

0.07, 0.38, 0.70, 0.74, 1.43	Northern Europe - AII Dossier 1995
0.75	Southern Europe - AII Dossier 1995

The resulting STMR and HR values are given below.

	N-EU	**S-EU**
STMR	0.74	0.709
HR	5.40	0.75

6.7.2.11 Animal matrices

Estimation of residues in livestock feed

A worst case diet was derived for different livestock species according to the table in Appendix G (Lundehn document 7031/VI/95 rev.4, July 1996). It is assumed that from each group of crops/commodities the item with the highest potential residue contribution on a dry matter basis is chosen. Then, the total diet is composed beginning with the group representing the highest contribution and filling the rest with feed from the other groups in descending order.
The evaluation is based on the following formula:

$$\text{Uptake [mg/kg bw/day]} = \frac{\text{Total intake of dry matter [kg/ animal/day]} \times \text{\% of diet} \times \text{Residue in feed item [mg/kg]}}{\text{Dry matter content of feed item [\%]} \times \text{Bodyweight [kg]}}$$

The following tables show the calculations of the maximum dietary burden for each relevant livestock species, which are based on the highest or median residue levels of bentazone, depending on the commodity. These maximum dietary burdens are then used to derive suitable MRLs for products of animal origin.

The table below summarises the residue values used for the dietary burden calculations and their origin. The maximum dietary burden of total bentazone residues for livestock was calculated using the highest residues (HR) or, if these were not applicable due to a bulk or processed commodity, the median residues (STMR / $STMR_P$).

Table 6.7/14 Residue values used for calculation of the feed burden

Crop	STMR / $STMR_P$ [mg/kg]	HR / HR_P [mg/kg]	Origin
Alfalfa forage	0.74	1.43	Residue trials alfalfa N-EU + S-EU
Maize forage	0.02	0.13	Residue trials maize N-EU + S-EU: bentazone
	0.47	1.79	Residue trials maize N-EU + S-EU: 6-OH-bentazone
	0.02	0.08	Residue trials maize N-EU + S-EU: 8-OH-bentazone
Maize forage	0.74	1.78	Residue trials maize N-EU + S-EU
Wheat / rye grain	0.06	--*	Residue trials wheat + barley N-EU + S-EU
Barley / oat grain	0.06	--*	Residue trials wheat + barley N-EU + S-EU
Maize grain	0.045	--*	Residue trials maize N-EU + S-EU
Wheat / barley bran	0.0552	--*	Rice mean processing factor of 0.92
Wheat / barley straw	0.12	0.65	Residue trials wheat + barley N-EU + S-EU
Peas (dry)	0.06	0.06	Residue trials dried beans N-EU + S-EU
Beans (dry)	0.06	0.06	Residue trials dried beans N-EU + S-EU
Lupins (dry)	0.06	0.06	Residue trials dried beans N-EU + S-EU
Potatoes	0.06	0.08	Residue trials potato N-EU + S-EU
Rape seed	0.06	--*	Residue trials linseed N-EU + S-EU
Rape seed meal	0.078	--*	Soybean mean processing factor of 1.3
Cotton seed	0.06	--*	Residue trials linseed N-EU + S-EU
Cotton seed meal	0.078	--*	Soybean mean processing factor of 1.3
Linseed	0.06	--*	Residue trials linseed N-EU + S-EU
Linseed meal	0.078	--*	Soybean mean processing factor of 1.3
Sunflower seed	0.06	--*	Residue trials linseed N-EU + S-EU
Sunflower seed meal	0.078	--*	Soybean mean processing factor of 1.3
Soybean	0.06	--*	Residue trials linseed N-EU + S-EU
Soybean meal	0.078	--*	Soybean mean processing factor of 1.3

* bulk / processed commodity

For cattle calculations were done with each of the relevant residues (bentazone, 6-OH- and 8-OH-bentazone, respectively) to get a more realistic picture of the actual dietary feed burden (for details please refer to M-II, 6.4).

Table 6.7/15 Estimated maximum dietary burden of bentazone residues for cattle

Crop	Dry Matter Content (%)	Residue Level (HR, mg/kg)	Dairy Cattle*		Beef Cattle**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Maize forage	20	0.13	100	0.023632	100	0.027852
Dietary burden:	mg/kg bw/day		0.024		0.028	
	mg/animal/day		13.0		9.7	
	mg/kg total feed (DM)		0.65		0.65	

* feed intake 20 kg DM, body weight (bw) 550 kg
** feed intake 15 kg DM, body weight (bw) 350 kg

Table 6.7/16 Estimated maximum dietary burden of 6-OH-bentazone residues for cattle

Crop	Dry Matter Content (%)	Residue Level (HR, mg/kg)	Dairy Cattle*		Beef Cattle**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Maize forage	20	1.79	100	0.325515	100	0.383570
Dietary burden:	mg/kg bw/day		0.33		0.38	
	mg/animal/day		179.0		134.2	
	mg/kg total feed (DM)		8.95		8.95	

* feed intake 20 kg DM, body weight (bw) 550 kg
** feed intake 15 kg DM, body weight (bw) 350 kg

Table 6.7/17 Estimated maximum dietary burden of 8-OH-bentazone residues for cattle

Crop	Dry Matter Content (%)	Residue Level (HR, mg/kg)	Dairy Cattle*		Beef Cattle**	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Maize forage	20	0.08	100	0.01454545	100	0.0171429
Dietary burden:	mg/kg bw/day		0.015		0.017	
	mg/animal/day		8.0		6.0	
	mg/kg total feed (DM)		0.40		0.40	

* feed intake 20 kg DM, body weight (bw) 550 kg
** feed intake 15 kg DM, body weight (bw) 350 kg

Table 6.7/18 Estimated maximum dietary burden of total bentazone residues for poultry and pigs

Crop	Dry matter content (%)	Residue level (HR)	Poultry		Pigs	
			% of diet	mg/kg bw/d	% of diet	mg/kg bw/d
I Green forage						
Maize forage	20	1.74	0	-	15	0.162000
II Grains						
Wheat grain	86	0.06	70	0.003084	5	0.000140
V Root + Tuber						
Potatoes	15	0.08	20	0.006737	60	0.012800
VI Oil seed						
Rape seed meal	86	0.078	10	0.000573	20	0.000726
Dietary burden:	mg/kg bw/day		0.010		0.176	
	mg/animal/day		0.020		13.17	
	mg/kg total feed (DM)		0.165		4.392	

Thus, the doses to be used when estimating the maximum residues in products of animal origin are for

dairy cattle	0.330, 0.024, 0.015 mg/kg bw/d (6-OH, bentazone and 8-OH)
beef cattle	0.384, 0.028 and 0.017 mg/kg bw/d (6-OH, bentazone and 8-OH)
poultry	0.010 mg/kg bw/d
pigs	0.176 mg/kg bw/d

It should be noted that the doses assume that the diet completely consists of plant material which had been treated with bentazone, considering worst case assumptions such as intake of the highest residue (HR).

Cattle products

In the three goat metabolism studies of bentazone, 6-OH- and 8-OH-bentazone which can serve as feeding studies in this case (see chapter 6.4), the nominal dose levels were 2 to 3 mg/kg bw/day. Thus, overdosing factors of 107, 5 and 188 (bentazone, 6-OH- and 8-OH-bentazone, respectively), based on the dietary burden for beef cattle as a worst case, have to be applied to the residues.

When samples of the three goat metabolism studies were analysed, overall residues in milk were 0.021-0.027 mg/kg. Application of the overdosing factors leads to an anticipated overall residue for total bentazone (sum of bentazone, 6-OH- and 8-OH-bentazone) of 0.004 mg/kg in milk.
In tissues, residues of 0.018-0.03, 0.118-0.6, 0.011-0.013 and 0.007-1.57 mg/kg were found in liver, kidney, muscle and fat for the three analytes, respectively. Application of the overdosing factors leads to anticipated residues of 0.003, 0.03, 0.002 and 0.02 mg/kg for total bentazone (sum of bentazone, 6-OH- and 8-OH-bentazone), respectively. Since feed burden calculations are conducted under various worst-case assumptions extrapolations give a very conservative estimate of the residues.

Poultry products
In the hen metabolism study, which can serve as a feeding study in this case (see chapter 6.4), the nominal dose level for total bentazone (bentazone, 6-OH- and 8-OH-bentazone) was 10 mg/kg bw/day. Thus, the overdosing factor of 500 (10 mg/kg bw/day / 0.02 mg/kg bw/day) has to be applied to the residues

When samples were analysed for total bentazone, residues in eggs were 0.20 mg/kg. Application of the overdosing factor leads to an anticipated residue of 0.0004 mg/kg in eggs.
In tissues, residues of 0.15, 0.06, 0.48, 0.41 and 1.46 mg/kg were found in subcutaneous fat, peritoneal fat, leg muscle, breast muscle and live, respectively. Application of the overdosing factor leads to anticipated residues of 0.0003, 0.0001, 0.001, 0.0008 and 0.0029 mg/kg, respectively.

Since the residue definition is proposed to be

> **Bentazone (sum of bentazone and the conjugates of 6-OH-bentazone and 8-OH-bentazone expressed as bentazone)**

and an overall LOQ of 0.03 mg/kg is supported for the sum of bentazone and the conjugates of 6-OH-bentazone and 8-OH-bentazone expressed as bentazone the default MRL should apply for all animal tissues, eggs and milk.

In conclusion, it is proposed establish EU MRLs for **animal tissues, eggs and milk** of **0.03 mg/kg**.

6.8 Proposed pre-harvest intervals, re-entry or withholding periods

6.8.1 Pre-harvest interval (in days) for each relevant crop

Crop/Group	PHI (days)
Potato	F
Onion	F
Sweet corn	28
Chive, mint	F
Beans, peas (fresh)	28
Beans, peas (dry)	F
Soybean	F
Maize, barley, wheat	F
Valerian (root)	F
Alfalfa	F
Foxglove	F

F fixed by approved use

6.8.2 Re-entry period (in days) for livestock, to areas to be grazed

A re-entry period for livestock does not need to be defined since bentazone is not intended to be used in areas to be grazed.

6.8.3 Re-entry period for man to crops, buildings or spaces treated

Re-entry is possible after the spray deposits on the crops have dried given the worker is wearing adequate work clothing. A risk assessment for the user can be found in chapter 3.10.2 of the Annex II dossier (1995). Furthermore, bentazone will be applied early in the growing season. Based on the application timing, re-entry exposure to workers is not likely to occur.

6.8.4 Withholding period (in days) for animals feedingstuffs

A withholding period is not necessary.

6.8.5 Waiting period between last application and sowing or planting

Bentazone is intended to be used as an early post-emergence herbicide. A waiting period for sowing or planting does not need to be defined.

6.8.6 Waiting periods between application and handling treated products

This is not relevant here since a post-harvest treatment is not intended.

6.8.7 Waiting period before sowing/planting succeeding crops

Due to the fact that no accumulation of bentazone or its degradation products were observed in the confined rotational crop study, no limitation concerning the succeeding crops is necessary (see chapter 6.6).

6.9 Estimation of exposure through diet and other means

Assessments of the potential chronic and acute dietary consumer risk resulting from exposure to residues of bentazone (BAS 351 H) were performed using the EFSA calculation model for acute and chronic consumer exposure (version 2, August 2008). The EFSA model was used since it considers all the different diets and all consumer groups in the EU.

The ADI and ARfD values for bentazone are summarised in the table below.

Table 6.9/1 Toxicological end-points - bentazone

End-Point	Value	Study	Safety Factor	Reference
Acceptable Daily Intake (ADI)	0.1 mg/kg bw/d	24-month rat feeding study	100	EU Review Report (2000), 7585/VI/97-final
Acute Reference Dose (ARfD)	0.25 mg/kg bw	90-day rat feeding study	100	EU Review Report (2000), 7585/VI/97-final

6.9.1 TMDI calculations

The values used in the dietary risk assessment in this chapter are listed in Table 6.9/2 below.

The chronic and acute consumer risk assessments (see Table 6.9/3 and Table 6.9/4) were performed using the MRLs for bentazone from the European Commission database (according to Regulation (EC) No. 396/2005, last amended by Regulation (EU) No. 893/2010) amended by the proposed MRLs listed in the EFSA Reasoned Opinion 2011, as indicated in Table 6.9/2 (MRL values[1]). In addition, new MRLs proposed in this dossier are used in the risk assessment (see /MRL changes[2] in Table 6.9/2). For all crops listed in the residues section of this dossier MRLs have been calculated on the basis of residue trials conducted or based on MRL values extrapolated according to the EC guidance documents 7525/VI/95 rev. 9 of March 2011 (see chapter 6.7).

Table 6.9/2 Maximum residue levels for bentazone[#] in the EU and changes proposed by BASF

Crop/Tissue	EU MRL/MRL changes[2]
1. FRUIT FRESH OR FROZEN; NUTS	0.1*/0.06*
2. VEGETABLES FRESH OR FROZEN	-
(i) Root and tuber vegetables	0.1*/-
(a) Potatoes	0.1*/0.15
(b) Tropical root and tuber vegetables	0.1*/0.15
(c) Other root and tuber vegetables except sugar beet	0.1*/0.06*
(ii) Bulb vegetables	0.1*/0.1
(iii) Fruiting vegetables	-
(a) Solanacea	0.1*/0.06*
(b) Cucurbits - edible peel	0.1*/0.06*
(c) Cucurbits-inedible peel	0.1*/0.06*
(d) Sweet corn	0.3/0.4
(e) Other fruiting vegetables	0.1*/0.06*
(iv) Brassica vegetables	0.1*/0.06*
(v) Leaf vegetables & fresh herbs	-
(a) Lettuce and other salad plants including Brassicacea	0.1*/0.06*
(b) Spinach & similar (leaves)	0.1*/0.06*
(c) Vine leaves (grape leaves)	0.1*/0.06*
(d) Water cress	0.1*/0.06*
(e) Witloof	0.1*/0.06*
(f) Herbs	10[1]
(vi) Legume vegetables (fresh)	-
Beans (with pods) (Green bean (french beans, snap beans), scarlet runner bean, slicing bean, yardlong beans)	0.3[1]
Beans (without pods) (Broad beans, Flageolets, jack bean, lima bean, cowpea)	0.05[1]/0.06
Peas (with pods) (Mangetout (sugar peas))	0.5
Peas (without pods) (Garden pea, green pea, chickpea)	0.2
Lentils	0.1*/0.06*
Others	0.1*/0.06*
(vii) Stem vegetables (fresh)	0.1*/0.06*
(viii) Fungi	0.1*/0.06*
(ix). Sea weeds	0.1*/0.06*
3. PULSES, DRY	0.1*/0.3
4. OILSEEDS AND OILFRUITS	0.1*/0.1
5. CEREALS	0.1*/-
Barley	0.1*/0.1
Buckwheat (Amaranthus, quinoa)	0.1*/0.06*
Maize	0.1*/0.1
Millet (Foxtail millet, teff)	0.1*/0.1
Oats	0.1*/0.1
Rice	0.1*/0.06*
Rye	0.1*/0.1
Sorghum	0.1*/0.1
Wheat (Spelt, triticale)	0.1*/0.1
Others	0.1*/0.06*

Table 6.9/2 Maximum residue levels for bentazone[#] in the EU and changes proposed by BASF

Crop/Tissue	EU MRL/MRL changes[2]
6. TEA, COFFEE, HERBAL INFUSIONS AND COCOA	0.1*/-
(i) Tea (dried leaves and stalks, fermented or otherwise of Camellia sinensis)	0.1*/0.06*
(ii) Coffee beans	0.1*/0.06*
(iii) Herbal infusions (dried)	0.1*/-
(a) Flowers	0.1*/0.06*
(b) Leaves	0.1*/0.06*
(c) Roots	0.1*/0.15
(d) Other herbal infusions	0.1*/0.06*
(iv) Cocoa (fermented beans)	0.1*/0.06*
(v) Carob (st johns bread)	0.1*/0.06*
7. HOPS (dried) , including hop pellets and unconcentrated powder	0.1*/0.06*
8. SPICES	0.1*/-
(i) Seeds	0.1*/0.06*
(ii) Fruits and berries	0.1*/0.06*
(iii) Bark	0.1*/0.06*
(iv) Roots or rhizome	0.1*/0.15
(v) Buds	0.1*/0.06*
(vi) Flower stigma	0.1*/0.06*
(vii) Aril	0.1*/0.06*
9. SUGAR PLANTS	0.1*/0.06*
10. PRODUCTS OF ANIMAL ORIGIN-TERRESTRIAL ANIMALS	-
(i) Meat, preparations of meat, offals, blood, animal fats fresh chilled or frozen, salted, in brine, dried or smoked or processed as flours or meals other processed products such as sausages and food preparations based on these	0.05*/0.03*
(ii) Milk and cream, not concentrated, nor containing added sugar or sweetening matter, butter and other fats derived from milk, cheese and curd	0.02*/0.03*
(iii) Birds eggs, fresh preserved or cooked Shelled eggs and egg yolks fresh, dried, cooked by steaming or boiling in water, moulded, frozen or otherwise preserved whether or not containing added sugar or sweetening matter	0.05*/0.03*

(*) indicates the lower limit of analytical determination
(#) sum of bentazone and the conjugates of 6-OH- and 8-OH-bentazone expressed as bentazone
(1) EFSA Reasoned Opinion, EFSA Journal 2011;9(5):2188
(2) MRL changes proposed by BASF

Table 6.9/3 TMDI calculation for total bentazone with EFSA PRIMo Model (rev 2.0) using EU MRLs and MRLs proposed by BASF

Bentazone			
Status of the active substance:	**Approved**	Code no.	
LOQ (mg/kg bw):	0.06	proposed LOQ:	
Toxicological end points			
ADI (mg/kg bw/day):	**0.1**	ARfD (mg/kg bw):	**0.25**
Source of ADI:	**Dir 00/68**	Source of ARfD:	**Dir 00/68**
Year of evaluation:	**2000**	Year of evaluation:	**2000**

Chronic risk assessment

	TMDI (range) in % of ADI minimum - maximum
	1 4
No of diets exceeding ADI:	--- 0

Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities	pTMRLs at LOQ (in % of ADI)
4.2	WHO Cluster diet B	0.9	Wheat	0.5	Herbs	0.4	FRUIT (FRESH OR FROZEN)	1.3
4.2	UK Toddler	1.4	SUGAR PLANTS	0.6	Milk and cream,	0.5	Potatoes	2.6
4.0	FR toddler	1.2	Milk and cream,	0.8	Potatoes	0.7	FRUIT (FRESH OR FROZEN)	2.4
3.9	NL child	0.9	FRUIT (FRESH OR FROZEN)	0.9	Potatoes	0.9	Milk and cream,	2.2
3.7	UK Infant	1.2	Milk and cream,	0.6	SUGAR PLANTS	0.5	Potatoes	2.4
3.6	DE child	1.4	FRUIT (FRESH OR FROZEN)	0.4	Herbs	0.4	Milk and cream,	2.2
3.6	IE adult	0.6	FRUIT (FRESH OR FROZEN)	0.5	Tropical root and tuber vegetables	0.4	Herbs	1.2
3.2	FR infant	0.9	FRUIT (FRESH OR FROZEN)	0.8	Milk and cream,	0.6	Potatoes	2.1
3.1	WHO cluster diet E	0.6	Potatoes	0.5	Herbs	0.4	Wheat	0.8
3.0	WHO cluster diet D	0.7	Herbs	0.7	Wheat	0.6	Potatoes	0.7
2.6	DK child	0.6	Wheat	0.4	Rye	0.4	Milk and cream,	1.1
2.5	SE general population 90th percentile	0.6	Potatoes	0.4	Milk and cream,	0.3	FRUIT (FRESH OR FROZEN)	1.2
2.5	WHO regional European diet	0.6	Potatoes	0.4	Herbs	0.3	Wheat	0.8
2.3	PT General population	0.8	Potatoes	0.4	Wheat	0.4	FRUIT (FRESH OR FROZEN)	0.7
2.2	ES child	0.4	Wheat	0.4	Milk and cream,	0.3	FRUIT (FRESH OR FROZEN)	1.1
2.0	WHO Cluster diet F	0.5	Potatoes	0.4	Wheat	0.2	FRUIT (FRESH OR FROZEN)	0.7
1.6	IT kids/toddler	0.7	Wheat	0.2	Herbs	0.2	FRUIT (FRESH OR FROZEN)	0.5
1.6	NL general	0.4	Potatoes	0.3	FRUIT (FRESH OR FROZEN)	0.2	Wheat	0.7
1.5	UK vegetarian	0.2	SUGAR PLANTS	0.2	Potatoes	0.2	Wheat	0.7
1.3	ES adult	0.2	FRUIT (FRESH OR FROZEN)	0.2	Wheat	0.1	Milk and cream,	0.7
1.3	UK Adult	0.2	SUGAR PLANTS	0.2	Potatoes	0.2	FRUIT (FRESH OR FROZEN)	0.7
1.3	FR all population	0.4	FRUIT (FRESH OR FROZEN)	0.3	Wheat	0.2	Potatoes	0.6
1.3	IT adult	0.4	Wheat	0.2	Herbs	0.2	FRUIT (FRESH OR FROZEN)	0.4
1.2	LT adult	0.5	Potatoes	0.1	FRUIT (FRESH OR FROZEN)	0.1	Milk and cream,	0.5
1.1	DK adult	0.2	Potatoes	0.2	Wheat	0.2	FRUIT (FRESH OR FROZEN)	0.5
1.0	PL general population	0.5	Potatoes	0.2	FRUIT (FRESH OR FROZEN)	0.1	Herbs	0.4
0.9	FI adult	0.2	Potatoes	0.2	Milk and cream,	0.2	FRUIT (FRESH OR FROZEN)	0.5

The chronic consumer risk assessment (see Table 6.9/3) based on established as well as newly proposed EU MRLs yielded ADI utilisation rates well below 100% with the EFSA PRIMo. TMDI calculations led to an ADI utilisation of 4.2% for the WHO Cluster diet B, with wheat and herbs contributing 0.9% and 0.5% to the ADI, respectively. ADI utilisation for UK Toddler was 4.2% with sugar plants and milk and cream contributing 1.4% and 0.6% to the ADI, respectively.

According to the presented TMDI calculations, a long-term intake of bentazone (sum of bentazone, 6-OH- and 8-OH-bentazone) residues is unlikely to present a public health concern, even when the worst case situation is considered.

6.9.2 NEDI calculations

For all models included in the EFSA model, the use of STMR or $STMR_P$ values in the estimation of the chronic dietary consumer risk is up to this point in time not necessary since the crude overestimated TMDI of bentazone was well below 100% of the ADI.

6.9.3 NESTI calculations

An assessment of the potential acute dietary consumer risk due to exposure to residues of total bentazone (see Table 6.9/4) was performed using the EFSA calculation model and the established EU MRLs as well as the proposed MRLs.

Table 6.9/4 NESTI calculation for total bentazone with EFSA PRIMo Model (rev 2.0) using EU MRLs and MRLs proposed by BASF

Acute risk assessment /children						Acute risk assessment / adults / general population					
The acute risk assessment is based on the ARfD. For each commodity the calculation is based on the highest reported MS consumption per kg bw and the corresponding unit weight from the MS with the critical consumption. If no data on the unit weight was available from that MS an average European unit weight was used for the IESTI calculation. In the **IESTI 1** calculation, the variability factors were 10, 7 or 5 (according to JMPR manual 2002), for lettuce a variability factor of 5 was used. In the **IESTI 2** calculations, the variability factors of 10 and 7 were replaced by 5. For lettuce the calculation was performed with a variabilty factor of 3. **Threshold MRL** is the calculated residue level which would leads to an exposure equivalent to 100 % of the ARfD.											
Unprocessed commodities	**No of commodities for which ARfD/ADI is exceeded (IESTI 1):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 1):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		---
	IESTI 1	***)**	****)**	**IESTI 2**	***)**	****)**	**IESTI 1**	***)**	****)**	**IESTI 2**	***)**	****)**
	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
	22.9	Celery leaves	10 / -	22.9	Celery leaves	10 / -	4.8	Parsley	10 / -	4.8	Parsley	10 / -
	11.7	Sweet corn	0.4 / -	8.4	Sweet corn	0.4 / -	3.5	Sweet corn	0.4 / -	2.5	Sweet corn	0.4 / -
	9.2	Potatoes	0.15 / -	6.6	Potatoes	0.15 / -	1.8	Potatoes	0.15 / -	1.4	Potatoes	0.15 / -
	5.2	Chervil	10 / -	5.2	Chervil	10 / -	1.3	Yams	0.15 / -	1.3	Pumpkins	0.06 / -
	3.6	Melons	0.06 / -	3.6	Melons	0.06 / -	1.3	Pumpkins	0.06 / -	1.1	Yams	0.15 / -
	No of critical MRLs (IESTI 1)			---			**No of critical MRLs (IESTI 2)**			---		
Processed commodities	**No of commodities for which ARfD/ADI is exceeded:**		---				**No of commodities for which ARfD/ADI is exceeded:**		---			
			*****)**						*****)**			
	Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)				Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)			
	1.2	Apple juice	0.06 / -				0.2	Orange juice	0.06 / -			
	1.2	Orange juice	0.06 / -				0.2	Bread/pizza	0.1 / -			
	1.0	Carrot, juice	0.06 / -				0.2	Apple juice	0.06 / -			
	0.8	Potato puree (flakes)	0.15 / -				0.1	Wine	0.06 / -			
	0.8	Grape juice	0.06 / -				0.1	Pineapples preserved	0.06 / -			
*) The results of the IESTI calculations are reported for at least 5 commodities. If the ARfD is exceeded for more than 5 commodities, all IESTI values > 90% of ARfD are reported. **) pTMRL: provisional temporary MRL ***) pTMRL: provisional temporary MRL for unprocessed commodity												

The acute dietary risk assessment performed for the use of bentazone in plant commodities and animal products using EU MRLs and new MRLs proposed by BASF according to the EFSA calculation model clearly indicates that there is no acute risk for any subpopulation group under consideration, the highest ARfD utilisation being 22.9% for children.

6.10 Other/special studies

Besides the crops targeted for human consumption, further residue studies on alfalfa and foxglove were performed. The summaries are presented below.

6.10.1 Alfalfa

Table 6.10/1 GAP on BAS 351 H for residue trials in/on alfalfa

Crop	Maximum Applied Dose	Water Volume	PHI	Application Method	Application Timing
Alfalfa	1 x 0.96 kg BAS 351 H/ha	200-400 L/ha	F	spray application	BBCH 12-13

PHI = pre-harvest interval

Table 6.10/2 GAP information of residue trials conducted in alfalfa in 2007

Region	Country	Formulation	Application[0]				DALA[1]
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
EU North	France (2 trials)	BAS 762 01 H, SL	spray application	0.600	0.150	1	14 23
EU South	Italy (2 trials) Spain (2 trials)	BAS 762 01 H, SL	spray application	0.600	0.150	1	28-41

0) actual application rates varied by 10% at most
1) days after last application

Table 6.10/3 GAP information of residue trials conducted in alfalfa before 2007 already reviewed in the context of Annex I inclusion

Region Doc ID	Country (Trial No.)	Formulation	Application[0]				DALA[1]	Total Bentazone Residues (mg/kg)
			Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.		
EU North 1994/10678	Sweden (35107H77/15E)	BAS 351 07 H	spray application	1.44	n.r.	1	262	0.74
	Sweden (35107H77/16E)	BAS 351 07 H	spray application	1.44	n.r.	1	58	0.38
	United Kingdom (35107H80/1E)	BAS 351 07 H	spray application	1.44	0.655	1	40	0.70
	United Kingdom (35107H80/2E)	BAS 351 07 H	spray application	1.44	0.655	1	40	1.43
	Sweden (35107H77/17E)	BAS 351 07 H	spray application	0.96	n.r.	2	32	0.07
EU South 1994/10678	Italy (35132H88/5A)	BAS 351 32 H	spray application	1.44	0.360	1	0 15 29 44	n.rel. n.rel. 0.75 n.rel.

0) actual application rates varied by 10% at most
1) days after last application
n.r. not reported
n.rel. not relevant

Report: II A 6.10/1
Kreke N. 2008(k)
BAS 762 01 H with adjuvant BAS 9047 0S (DASH HC) - Residues at harvest of Imazamox and Bentazone in alfalfa (RAC green matter and hay) following one treatment with a tankmix from three open field trials, Northern France, Italy, Spain, 2007
BASF DocID 2007/1028360
Guidelines: EEC 96/68; EEC 7029/VI/95 rev. 5
GLP: Yes
(laboratory certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report: II A 6.10/2
Kreke N. 2010(u)
Amendment: Residues at harvest of Imazamox, Bentazone in alfalfa (RAC green matter, hay) following one treatment with a tankmix of BAS 762 01 H with adjuvant BAS 9047 0S (DASH HC), three open field trials, Northern France, Italy Spain 2007
BASF DocID 2010/1155809
Guidelines: EEC 96/68; EEC 7029/VI/95 rev. 5
GLP: Yes
(laboratory certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Executive Summary

During the 2007 growing season, three field trials were conducted in Northern and Southern Europe to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC). The tankmix of BAS 762 01 H, an SL formulation of bentazone and imazamox, and the adjuvant BAS 9047 0S (DASH HC) was foliar applied once at a rate equivalent to 0.600 kg a.s./ha of bentazone to alfalfa targeted at a crop growth stage BBCH 12-19. In the French trial, the growth stage at application was BBCH 37. Data on imazamox is not reported any further since it is not relevant for this dossier. Specimens of alfalfa green matter and hay were collected between 14-41 and 23-41 days after the application, respectively.
All samples were analysed according to BASF Method No. 438/2. The limit of quantitation was 0.01 mg/kg for all analytes. Total bentazone residues in alfalfa green matter were in a range of 0.17-5.40 mg/kg at harvest.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 762 01 H (SL), BAS 9047 0S
Lot/Batch #:	402009; bentazone (BAS 351 H) 480 g/L, imazamox 22.4 g/L nominal
Purity:	Not reported
CAS#:	25057-89-0
Development code:	Not reported
Spiking levels:	0.01-1.0 mg/kg

2. Test Commodity:

Crop:	Alfalfa (lucerne)
Type:	Legume vegetables
Variety:	Symphonie, Emilliana, Brago
Botanical name:	*Medicago sativa*
Crop parts(s) or processed commodity:	Green matter, hay
Sample size:	Min. 0.5-2 kg / 12 units (nominal)

B. STUDY DESIGN

1. Test procedure

During the 2007 growing season, three field trials were conducted in Northern France, Italy and Spain to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
The tankmix of BAS 762 01 H, an SL formulation of bentazone (480 g/L) and imazamox (22.4 g/L), and the adjuvant BAS 9047 0S (DASH HC) was foliar applied once at a rate equivalent to 0.600 kg a.s./ha of bentazone to alfalfa targeted at a crop growth stage BBCH 12-19. In the French trial, the growth stage at application was BBCH 37. The nominal spray volume used was 100-400 L/ha.
Specimens of alfalfa green matter and hay were collected between 14-41 and 23-41 days after the application, respectively. Control samples were taken from each trial. Samples were stored deep-frozen until analysis. The maximum storage interval from sampling until analysis was about eight months.

Table 6.10/5 Target application rates and timings for alfalfa

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2007	3	1	F	BAS 762 01 H (SL)	Bentazone Imazamox	0.600 0.028	100-400	BBCH 12-19

2. Description of analytical procedures

All samples were analysed according to BASF Method No. 438/2. Bentazone, 6-OH-bentazone and 8-OH-bentazone are extracted from plant material using a mixture of methanol and water. The two metabolites that were present as glucosides were hydrolysed using enzymatic cleavage to 6-OH-bentazone and 8-OH-bentazone. After a $Ca(OH)_2$ precipitation step to remove acidic plant constituents, a reversed phase C_{18}-column clean-up was performed or the extract was filtered and one aliquot taken, evaporated to dryness and dissolved in a methanol/water solution. The final determination of the residues of bentazone and its hydroxyl metabolites was performed by LC-MS/MS. The limit of quantitation was 0.01 mg/kg for all analytes.

II. RESULTS AND DISCUSSION

The residue ranges are presented in Table 6.10/4. Details can be found in Table 6.10/5 and Table 6.10/6.
In all samples of treated alfalfa green matter specimens, residues of bentazon and its metabolite 6-OH-betazone were found at levels ranging from 0.012-0.073 mg/kg and 0.137-5.646 mg/kg, respectively. In hay, residues of those two compounds were 0.070-0.230 mg/kg and 0.635-3.492 mg/kg, respectively.
Residues of metabolite 8-OH-bentazone were only found in the Northern European trial at a level of 0.034 mg/kg for green matter and 0.042 mg/kg for hay.
No residues of bentazone and its metabolites above the LOQ were found in the control samples.

Table 6.10/6: Summary of residues in alfalfa

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	2007	14	49	green matter	0.073	5.646	0.034	5.40
		23	**n.r.**	**hay**	**0.230**	**3.492**	**0.042**	**3.54**
EU South		**28-41**	**65-73**	**green matter**	**0.012-0.033**	**0.137-0.249**	**<0.01**	**0.17-0.25**
		30-41	**n.r.**	**hay**	**0.070-0.075**	**0.653-0.680**	**<0.01**	**0.70-0.72**

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Total bentazone residues in alfalfa green matter were in a range of 0.17-5.40 mg/kg at harvest.

Table 6.10/7 Residues of bentazone in alfalfa after one application of BAS 762 01 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	B44122	Alfalfa	France (North)	BAS 762 01 H: 1 x 0.600	37	14	green matter	0.073	5.646	0.034	*5.40*	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2007/1028360					23	hay	0.230	3.492	0.042	3.54	Bentazone				
Trial No.	A/NF/H/07/169											green matter	0.01-0.10	4	105	3.9
GLP:	yes											hay	0.01-0.10	4	101	8.9
Year:	2007											6-OH-bentazone				
												green matter	0.01-1.0	4	79	12
												hay	0.01-0.10	3	81	22
												8-OH-bentazone				
												green matter	0.01-0.10	4	101	12
												hay	0.01-0.10	3	105	11

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
6) at application
values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.10/8 Residues of bentazone in alfalfa after one application of BAS 762 01 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	B44122	Alfalfa	Italy	BAS 762 01 H: 1 x 0.600	11-13	41	green matter	0.033	0.137	<0.01	0.17	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2007/1028360					41	hay	0.070	0.653	<0.01	*0.70*	Bentazone				
Trial No.	A/IT/H/07/170											green matter	0.01-0.10	4	105	3.9
GLP:	yes											hay		4	101	8.9
Year:	2007											6-OH-bentazone				
Study code:	B44122	Alfalfa	Spain	BAS 762 01 H: 1 x 0.600	12-19	28	green matter	0.012	0.249	<0.01	0.25	green matter	0.01-1.0	4	79	12
Doc ID:	2007/1028360					30	hay	0.075	0.680	<0.01	*0.72*	hay		3	81	22
Trial No.	A/SP/H/07/171											8-OH-bentazone				
GLP:	yes											green matter	0.01-0.10	4	101	12
Year:	2007											hay		3	105	11

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
6) at application
values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Report:	II A 6.10/3 Kreke N. 2008(l) BAS 762 AA H - Determination of residues at harvest of Imazamox and Bentazone in alfalfa (RAC green matter and hay) following one treatment with BAS 762 AA H (22.4/480 g/L) from three open field trials in Northern and Southern Europe, 2007 BASF DocID 2007/1023135
Guidelines:	EEC 96/68; EEC 7029/VI/95 rev. 5
GLP:	Yes (laboratory certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	II A 6.10/4 Kreke N. 2008(m) First amendment: Determination of residues at harvest of Imazamox and Bentazone in alfalfa (RAC green matter and hay) following one treatment with BAS 762 AA H (22.4/480 g/L) from three open field trials, Northern and Southern Europe, 2007 BASF DocID 2008/1097982
Guidelines:	EEC 96/68; EEC 7029/VI/95 rev. 5
GLP:	Yes (laboratory certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Report:	II A 6.10/5 Kreke N. 2010(v) Second amendment: Determination of residues at harvest of Imazamox and Bentazone in alfalfa (RAC green matter, hay) following one treatment with BAS 762 01 H (22.4/480 g/L) from three open field trials in Northern and Southern Europe, 2007 BASF DocID 2010/1155808
Guidelines:	EEC 96/68; EEC 7029/VI/95 rev. 5
GLP:	Yes (laboratory certified by Swiss Federal Office of Public Health, Berne, Switzerland)

Executive Summary

During the 2007 growing season, three field trials were conducted in Northern and Southern Europe to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC). The product BAS 762 01 H, an SL formulation of bentazone and imazamox, was foliar applied once at a rate equivalent to 0.600 kg a.s./ha of bentazone to alfalfa targeted at a crop growth stage BBCH 12-19. Data on imazamox is not reported any further since it is not relevant for this dossier. Specimens of alfalfa green matter and hay were collected between 14-41 and 23-41 days after the application, respectively.
All samples were analysed according to BASF Method No. 438/2. The limit of quantitation was 0.01 mg/kg for all analytes. Total bentazone residues in alfalfa green matter were in a range of 0.19-1.42 mg/kg at harvest.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 762 01 H (SL)
Lot/Batch #:	402009; bentazone (BAS 351 H) 480 g/L, imazamox 22.4 g/L nominal
Purity:	Not reported
CAS#:	25057-89-0
Development code:	Not reported
Spiking levels:	0.01-0.10 mg/kg

2. Test Commodity:

Crop:	Alfalfa (lucerne)
Type:	Legume vegetables
Variety:	Symphonie, Emiliana, Arago
Botanical name:	*Medicago sativa*
Crop parts(s) or processed commodity:	Green matter, hay
Sample size:	Min. 0.5-2 kg / 12 units (nominal)

B. STUDY DESIGN

1. Test procedure

During the 2007 growing season, three field trials were conducted in Northern France, Italy and Spain to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).

The product BAS 762 01 H, an SL formulation of bentazone (480 g/L) and imazamox (22.4 g/L), was foliar applied once at a rate equivalent to 0.600 kg a.s./ha of bentazone to alfalfa targeted at a crop growth stage BBCH 12-19. The nominal spray volume used was 100-400 L/ha.

Specimens of alfalfa green matter and hay were collected between 14-41 and 23-41 days after the application, respectively. Control samples were taken from each trial. Samples were stored deep-frozen until analysis. The maximum storage interval from sampling until analysis was about three months.

Table 6.10/9 Target application rates and timings for alfalfa

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
2007	3	1	F	BAS 762 01 H (SL)	Bentazone Imazamox	0.600 0.028	100-400	BBCH 12-19

2. Description of analytical procedures

All samples were analysed according to BASF Method No. 438/2 (L0044/02). Bentazone, 6-OH-bentazone and 8-OH-bentazone are extracted from plant material using a mixture of methanol and water. After removing hydrophobic compounds with iso-octane by liquid-liquid partition and complete evaporation of organic solvent, potential conjugates of the analytes were decomposed by enzymatic treatment. After a final clean-up by solid phase extraction on a C_{18}-SPE cartridge, residues of bentazone, 6-OH-bentazone and 8-OH-bentazone were detemined by mass spectrometric detection after liquid chromatographic separation (HPLC-MS/MS). The limit of quantitation was 0.01 mg/kg for all analytes.

II. RESULTS AND DISCUSSION

The residue ranges are presented in Table 6.10/8. Details can be found in Table 6.10/9 and Table 6.10/10.
In all samples of treated alfalfa green matter specimens, residues of bentazone and its metabolite 6-OH-betazone were found at levels ranging from 0.014-0.059 and 0.169-1.439 mg/kg, respectively. In hay, residues of those two compounds were 0.041-0.118 mg/kg and 0.502-1.309 mg/kg, respectively.
Residues of metabolite 8-OH-bentazone were only found in the Northern European trial at a level of 0.011 mg/kg for green matter.
No residues of bentazone and its metabolites above the LOQ were found in the control samples.

Table 6.10/10 Summary of residues in alfalfa

Region	Year	DALA[1]	Growth Stage (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Bentazone[2]	8-OH-Bentazone[3]	Total Bentazone[4]
EU North	2007	14	49	green matter	0.059	1.439	0.011	1.419
		23	**n.r.**	**hay**	**0.097**	**1.309**	**<0.01**	**1.329**
EU South		**28-41**	**65-73**	**green matter**	**0.014-0.030**	**0.169-0.436**	**<0.01**	**0.190-0.427**
		30-41	**n.r.**	**hay**	**0.041-0.118**	**0.502-0.708**	**<0.01**	**0.594-0.709**

1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938

Emboldened DALAs represent the intended pre-harvest interval

III. CONCLUSION

Total bentazone residues in alfalfa green matter were in a range of 0.19-1.42 mg/kg at harvest.

Table 6.10/11 Residues of bentazone in alfalfa after one application of BAS 762 01 H in Northern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	B42952	Alfalfa	France (North)	BAS 762 01 H: 1 x 0.600	37	14	green matter	0.059	1.439	0.011	*1.419*	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2007/1023135					23	hay	0.097	1.309	<0.01	1.329	Bentazone				
Trial No.	A/NF/H/07/146											green matter	0.01-0.10	4	109	4.9
GLP:	yes											hay	0.01-0.10	4	99	4.4
Year:	2007											6-OH-bentazone				
												green matter	0.01-0.10	4	84	8.1
												hay	0.01-0.10	3	84	22
												8-OH-bentazone				
												green matter	0.01-0.10	4	88	5.1
												hay	0.01-0.10	3	89	1.7

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
6) at application
values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

Table 6.10/12 Residues of bentazone in alfalfa after one application of BAS 762 01 H in Southern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[6] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Bentazone	6-OH-Benta-zone[2]	8-OH-Benta-zone[3]	Total Benta-zone[4]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	B42952	Alfalfa	Italy	BAS 762 01 H: 1 x 0.600	11-13	41	green matter	0.030	0.169	<0.01	0.190	**Method:** No. 438/2; LOQ = 0.01 mg/kg				
Doc ID:	2007/1023135					41	hay	0.118	0.502	<0.01	*0.594*	Bentazone				
Trial No.	A/IT/H/07/147											green matter	0.01-0.10	4	99	4.9
GLP:	yes											hay	0.01-0.10	4	99	4.4
Year:	2007											6-OH-bentazone				
Study code:	B42952	Alfalfa	Spain	BAS 762 01 H: 1 x 0.600	12-19	28	green matter	0.014	0.436	<0.01	0.427	green matter	0.01-0.10	4	84	8.1
Doc ID:	2007/1023135					30	hay	0.041	0.708	<0.01	*0.709*	hay	0.01-0.10	3	84	22
Trial No.	A/SP/H/07/148											8-OH-bentazone				
GLP:	yes											green matter	0.01-0.10	4	88	5.1
Year:	2007											hay	0.01-0.10	3	89	1.7

0) actual application rates varied by 10% at most
1) days after last application
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
for calculation purposes, residues were treated as follows: 0.01 + 0.01 + 0.01 = 0.03; 0.01 + <0.01 + <0.01 = 0.03; <0.01 + <0.01 + <0.01 = <0.03
6) at application

values in *italic* were used for HR and STMR determination (needed for feed burden calculation)

6.10.2 Foxglove

Table 6.10/13 GAP on BAS 351 H for residue trials in/on foxglove

Crop	Maximum Applied Dose	Water Volume	PHI	Application Method	Application Timing
Woolly foxglove	1 x 0.960 kg BAS 351 H/ha	200-400 L/ha	F	spray application	BBCH 11-14

PHI = pre-harvest interval

Table 6.10/14 GAP information of residue trials conducted in foxglove during 2001-2003

Crop	Region	Country	Formulation	Application[0]				DALA[1]
				Method	Rate (kg a.s./ha)	Spray Conc. (kg a.s./hL)	No.	
Foxglove	EU North	Germany (5 trials)	Basagran: 480 g/L Bentazone	spray application	0.960	0.24-0.32	1	131-145

0) actual application rates varied by 10% at most
1) days after last application

Report: II A 6.10/6
Anonymous 2009(b)
Residue behaviour of Bentazon in/on floxglove, outdoor after application of Basagran (SL 480 g/L) in Germany, 1995-1998
BASF DocID 2008/1090967

Guidelines: None

GLP: Yes
(laboratory certified by Ministerium fuer Raumordnung, Landwirtschaft und Umwelt des Landes Sachsen-Anhalt, Magdeburg, Germany)

Executive Summary

During the growing seasons 1995-1998, 5 field trials were conducted in foxglove on different trials sites in Germany to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
Therefore, the formulated product Basagran (480 g/L bentazone, SL) was applied once at a rate equivalent to 0.960 kg a.s./ha of bentazone in a spray volume of 300-400 L/ha. Each trial consisted of a control (untreated) and a treated plot. The application was done at growth stages BBCH 10-12. Specimens of foxglove leaves were collected at growth stages BBCH 49, 131-145 days after the last application (DALA).
The leaves were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone using the analytical methods GLP-WF 1 or GLP-AG 2 (in one trial: 02/98 VG5), based on the modified BASF Method No. 197 (1982), which allows the determination of the analytes by GC-PFPD (Gas Chromatograph-Pulsed Flame Photometric Detector). The limit of quantitation (LOQ) of the method GLP-WF 1 is 0.019 mg/kg for bentazone or 0.024 mg/kg and 0.026 mg/kg for the metabolites 6-OH-bentazone and 8-OH-bentazone, respectively. The LOQ of the method GLP-AG 2 is 0.062 mg/kg for bentazone, 0.054 mg/kg and 0.039 mg/kg for 6-OH-bentazone and 8-OH-bentazone expressed as parent equivalents, respectively.
In foxglove leaf specimens, no residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone above the limit of quantitation were found in all specimens investigated.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material:

Description:	BAS 351 32 H, Basagran (SL)
Lot/Batch #:	Not reported, Bentazone: 480 g/L bentazone, nominal
Purity:	Not reported
CAS#:	25057-89-0
Development code:	Not reported
Spiking levels:	0.08-0.24 mg/kg

2. Test Commodity:

Crop:	Foxglove
Type:	Not specified
Variety:	Not reported
Botanical name:	*Digitalis lanata*
Crop parts(s) or processed commodity:	Leaves dried
Sample size:	0.2 kg

B. STUDY DESIGN

1. Test procedure

During the growing seasons 1995-1998, 5 field trials were conducted on foxglove on different trial sites in Germany to determine the residue level of bentazone (BAS 351 H) and its metabolites 6-OH-bentazone and 8-OH-bentazone in or on raw agricultural commodities (RAC).
Therefore, the formulated product Basagran (480 g/L bentazone, SL) was applied once at a rate equivalent to 0.960 kg a.s./ha of bentazone in a spray volume of 300-400 L/ha. Each trial consisted of a control (untreated) and a treated plot. The application was done at growth stages BBCH 10-12. Specimens of foxglove leaves were collected at growth stages BBCH 49, 131-145 days after the last application (DALA).

Table 6.10/15 Target application and timings for foxglove

Year	No. of Trials	No. of Appl.	F P or G	Test Item	Active Substance	Appl. Rate (kg a.s./ha)	Water Volume (L/ha)	Target Date/ Timing
1995-1998	5	1	F	Basagran (SL)	Bentazone	0.960	300-400	BBCH 10-12

2. Description of analytical procedures

The leaves were analysed for bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone using the analytical method GLP-WF 1 and GLP-AG 2 (without acetonitrile-hexane partition), based on the modified BASF Method No. 197 (1982), which allows the determination of the analytes by GC-PFPD (Gas Chromatograph-Pulsed Flame Photometric Detector). The limit of quantitation (LOQ) of the method GLP-WF 1 is 0.019 mg/kg for bentazone or 0.024 mg/kg and 0.026 mg/kg for the metabolites 6-OH-bentazone and 8-OH-bentazone, respectively. The LOQ of the method GLP-AG 2 is 0.062 mg/kg for bentazone, 0.039 mg/kg and 0.054 mg/kg for 6-OH-bentazone and 8-OH-bentazone expressed as parent equivalents, respectively.
Residues were extracted from foxglove specimens with methanol, followed by acetonitrile-hexane partition and hydrolysis with methanolic hydrochloric acid. After a liquid-liquid partition against dichloromethane and methylation with iodomethane, a clean-up on a florisil mini-column was carried out. The final determination was performed by GC-PFPD.

II. RESULTS AND DISCUSSION

The summary of residues in foxglove leaves is shown in Table 6.10/14, detailed residue levels are shown in Table 6.10/15.
No residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone above the limit of quantitation were found in all foxglove leaf specimens investigated.

Table 6.10/16 Summary of residues in foxglove

Region	Year	DALA[1]	Growth Stage[2] (BBCH)	Range of Residues (mg/kg)				
				Matrix	Bentazone	6-OH-Benta-zone[3]	8-OH-Benta-zone[4]	Total Benta-zone[5]
EU North	1995	131	49	dried leaves	<0.019	<0.024	<0.026	<0.066
	1996	136			<0.019	<0.024	<0.026	<0.066
	1997	141			<0.019	<0.024	<0.026	<0.066
	1998	145			<0.062	<0.039*	<0.054*	<0.155

1) days after last application
2) growth stage at sampling
2) expressed as 6-OH-bentazone
3) expressed as 8-OH-bentazone
4) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938
* expressed as parent equivalents

III. CONCLUSION

No residues of bentazone and its metabolites 6-OH-bentazone and 8-OH-bentazone above the limit of quantitation were found in all foxglove leaf specimens investigated.

Table 6.10/17 Residues of bentazone in foxglove leaves after one application of Basagran (48% bentazone, SL) in Nouthern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Benta-zone	6-OH-Benta-zone[3]	8-OH-Benta-zone[4]	Total Benta-zone[5]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	13216 LÜHWF 01/95	Foxglove	Germany	Basagran: 1 x 0.960	12	131	leaves (dried)	<0.019	<0.024	<0.026	<0.066	**Method:** GLP-WF 1 (based on BASF Method No. 187 (1982))				
Doc ID:	2008/1090967											LOQ = 0.019, 0.024, 0.026 mg/kg (bentazone, 6-OH-bent., 8-OH-bent.)				
Trial No	VG11											bentazone				
GLP:	yes											leaves	0.08-0.24	n.r.	50-73	18-20
Year	1995											6-OH-bentazone				
Study code:	20907 LÜHWF 01/96	Foxglove	Germany	Basagran: 1 x 0.960	10	136	leaves (dried)	<0.019	<0.024	<0.026	<0.066	leaves	0.08	n.r.	74-75	17-30
Doc ID:	2008/1090967											8-OH-bentazone				
Trial No	VG7											leaves	0.08	n.r.	69-74	16-27
GLP:	yes															
Year	1996															
Study code:	13205 LÜHWF 02/97	Foxglove	Germany	Basagran: 1 x 0.960	10-11	145	leaves (dried)	<0.019	<0.024	<0.026	<0.066					
Doc ID:	2008/1090967															
Trial No	VG3															
GLP:	yes															
Year	1997															
Study code:	15693 LÜHWF 01/97	Foxglove	Germany	Basagran: 1 x 0.960	10-11	141	leaves (dried)	<0.019	<0.024	<0.026	<0.066					
Doc ID:	2008/1090967															
Trial No	VG4															
GLP:	yes															
Year	1997															

Table 6.10/17 Residues of bentazone in foxglove leaves after one application of Basagran (48% bentazone, SL) in Nouthern Europe

Trial Details		Crop	Country	Formulation Application Rate[0] (kg a.s./ha)	Crop Growth Stage[2] (BBCH)	DALA[1]	Residues Found (mg/kg)					Recovery Data				
							Matrix	Benta-zone	6-OH-Benta-zone[3]	8-OH-Benta-zone[4]	Total Benta-zone[5]	Matrix	Fortifi-cation Range (mg/kg)	n	Mean (%)	RSD (%)
Study code:	13229	Foxglove	Germany	Basagran: 1 x 0.960	10	145	leaves (dried)	<0.062	<0.039*	<0.054*	<0.155	**Method:** GLP-AG 2 (based on BASF Method No.187 (1982))				
	LÜHWF 02/98											LOQ = 0.062, 0.054, 0.039 mg/kg (bentazone, 8-OH, 6-OH-bentazone)				
Doc ID:	2008/1090967											bentazone				
Trial No	VG5											leaves	0.1-0.2	10	87	8
GLP:	yes											6-OH-bentazone				
Year	1998											leaves	0.1-0.2	10	92	5
												8-OH-bentazone				
												leaves	0.1-0.2	10	109	8

0) actual application rates varied by 10% at most
1) days after last application
2) growth stage at last application
3) expressed as 6-OH-bentazone
4) expressed as 8-OH-bentazone
5) for total bentazone, metabolites were calculated as parent equivalent; the conversion factor for both metabolites is 0.938;
n.r. not reported
* expressed as parent equivalents

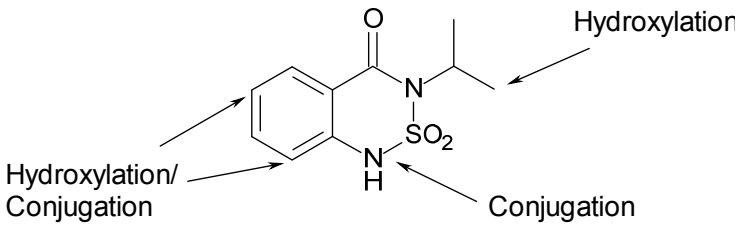
6.11 Summary and evaluation of residue behaviour and reasonable grounds

6.11.1 Summary and evaluation of residue behaviour

6.11.1.1 General

The observed metabolic transformations in plants and animals are summarised in Figure 6.11/1.

Figure 6.11/1 Targets of metabolic transformations on bentazone in plants and animals

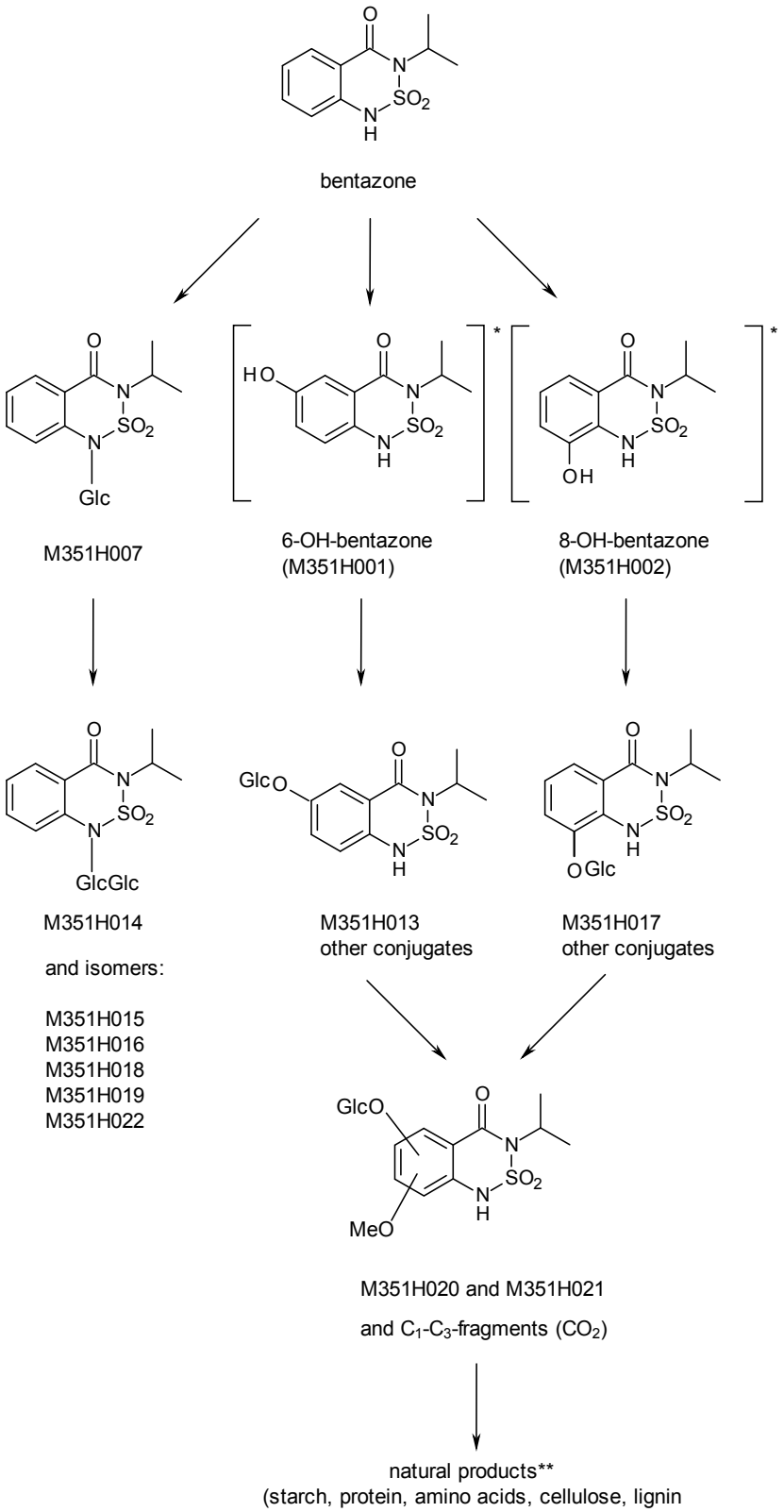


Bentazone is mainly metabolised by three key transformation steps:

- Hydroxylation mainly at the 6- and 8-position
- Conjugation at the nitrogen atom in 1-position and after oxidation in 6- and 8-position
- Degradation to C_1-C_2 pool with subsequent incorporation into natural products (such as starch, protein, amino acids, cellulose and lignin)

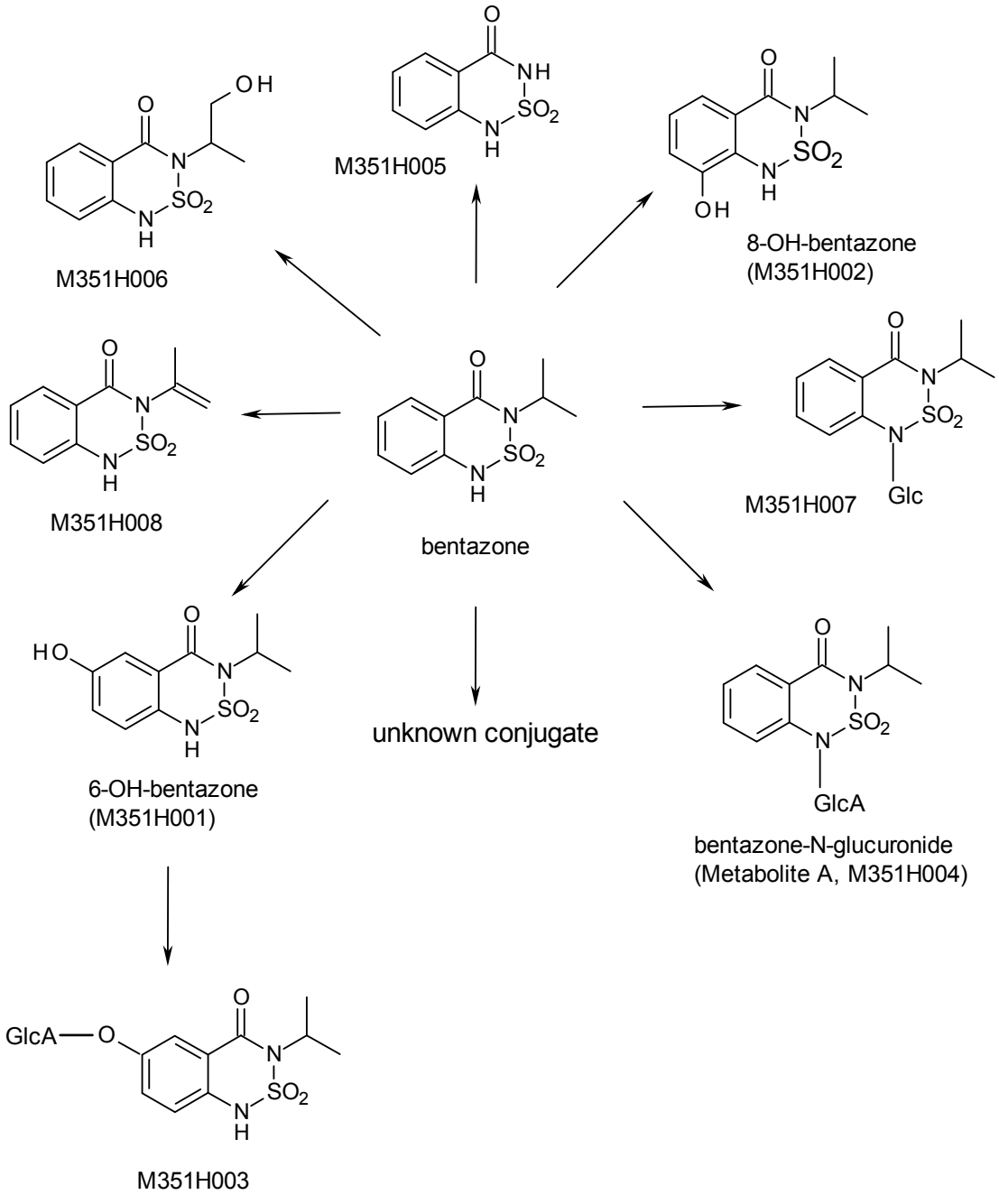
The metabolic pathways for bentazone in plants and animals are shown in Figure 6.11/2 and Figure 6.11/3.

Figure 6.11/2 Proposed metabolic pathway of bentazone in plants (potato, soybean, rice, maize, wheat, green bean)



* not detected as free metabolites
** do not represent "bound residues", as by IUPAC definition

Figure 6.11/3 Metabolic pathway of bentazone in animals (rat, goat and hen)



The metabolites identified in the different metabolism studies are summarised in Table 6.11/1.

Table 6.11/1: Summary of relevant identified bentazone and metabolites in plants and animals

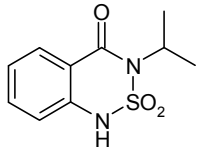
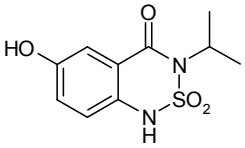
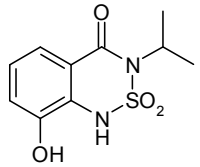
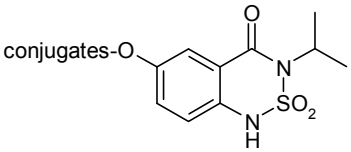
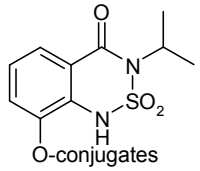
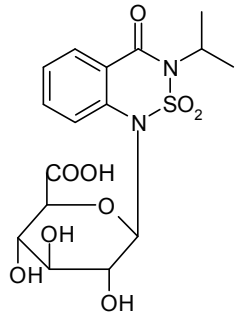
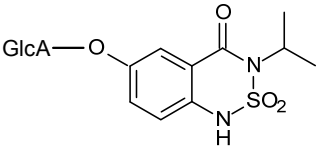
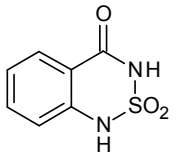
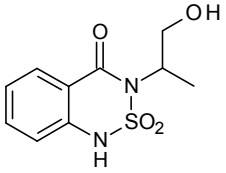
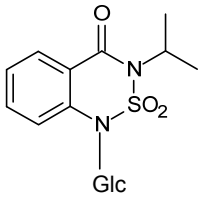
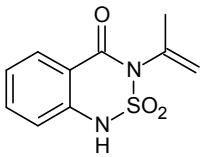
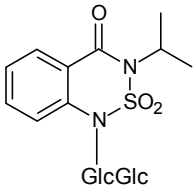
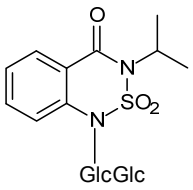
Code Name	Chemical Name	Metabolite Identity	Matrix
Bentazone Parent	3-Isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide		Potato Rice Maize Soybean Wheat Hen Goat Rat
6-OH-bentazone (M351H001)	3-Isopropyl-1H-2,1,3-benzothiadiazine-4(3H)-one-6-hydroxy-2,2-dioxide		Hen (excreta) Goat (urine) Rat
8-OH-bentazone (M351H002)	3-Isopropyl-1H-2,1,3-benzothiadiazine-4(3H)-one-8-hydroxy-2,2-dioxide		Soybean Goat (after 8-OH-bentazone dosing) Rat
Bentazone-6-O-glucoside (M351H013)	-		Potato Maize Rice Soybean Wheat
Bentazone-8-O-glucoside (M351H017)	-		Soybean Wheat
Bentazone-N-glucuronide (Metabolite A, M351H004)	3-isopropyl-1-methyl-2,2-dioxo-2,1,3-benzothiadiazin-4-one		Hen Goat Rat (urine)
M351H003	3-isopropyl-6-methoxy-2,2-dioxo-1H-2,1,3-benzothiadiazin-4-one		Rat (urine)

Table 6.11/1: Summary of relevant identified bentazone and metabolites in plants and animals

Code Name	Chemical Name	Metabolite Identity	Matrix
M351H005	2,2-dioxo-1H-2,1,3-benzothiadiazin-4-one		Rat (urine)
M351H006	3-(2-hydroxy-1-methyl-ethyl)-2,2-dioxo-1H-2,1,3-benzothiadiazin-4-one		Rat (faeces)
M351H007	3-isopropyl-1-methyl-2,2-dioxo-2,1,3-benzothiadiazin-4-one		Rat (urine)
M351H008	3-isopropenyl-2,2-dioxo-1H-2,1,3-benzothiadiazin-4-one		Rat (urine)
M351H014 and isomers: M351H015-016, -018-019, -022	-		Wheat
M351H020 and M351H021	-		Wheat

6.11.1.2 Proposed residue definition

The metabolism studies of bentazone have been evaluated by the German Rapporteur Member State in context of the Annex I inclusion procedure. The list of EU endpoints, given in Regulation (EC) No 396/2005 as amended by Regulation (EU) No 893/2010, is summarised below. Also, the proposed residue definitions are presented. For animal matrices, it is proposed to set the same residue definition as for plant matrices.

Table 6.11/2 EU End-points - bentazone

Endpoint	Active Substance: Bentazone	
	EU Endpoints Regulation (EC) No 396/2005 as amended by Regulation (EU) No 893/2010	Proposed Endpoints
Residue definition in plant matrices for risk assessment	Bentazone (sum of bentazone and the conjugates of 6-OH and 8-OH-bentazone expressed as bentazone)	Bentazone (sum of bentazone and the conjugates of 6-OH and 8-OH-bentazone expressed as bentazone)
Residue definition in plant matrices for monitoring	Bentazone (sum of bentazone and the conjugates of 6-OH and 8-OH-bentazone expressed as bentazone)	Bentazone (sum of bentazone and the conjugates of 6-OH and 8-OH-bentazone expressed as bentazone)
Residue definition in animal matrices for risk assessment	Bentazone	Bentazone (sum of bentazone and the conjugates of 6-OH and 8-OH-bentazone expressed as bentazone)
Residue definition animal matrices for monitoring	Bentazone	Bentazone (sum of bentazone and the conjugates of 6-OH and 8-OH-bentazone expressed as bentazone)

Plant

Metabolism studies were carried out in six crops belonging to three different crop categories:

For root and tuber vegetables:	potato
For pulses and oilseeds	green beans and soybean
For cereals:	maize, rice and wheat

Except for the wheat study plant metabolism studies were considered appropriate in the initial evaluation. Bentazone and the glucose conjugates of 6-OH- and 8-OH-bentazone are the main residues. No further metabolite exceeded 10% TRR. Furthermore, complete degradation of bentazone to natural products such as sugar/starch, lignin and protein was described as main metabolic route.
To confirm results of the five initially submitted metabolism studies with state of the art technology, it was agreed with former RMS to conduct one further metabolism study in wheat. The wheat metabolism study confirmed results of the previous studies with bentazone and the glucose conjugates of 6-OH- and 8-OH-bentazone as main residues. Also complete degradation of bentazone to natural products such as sugar/starch, lignin and protein was confirmed as main metabolic route.
Therefore, the metabolism of bentazone in plants is well characterised and the sum of bentazone and the conjugates of 6-OH- and 8-OH-bentazone expressed as bentazone equivalents were defined as components of the food residue.

Animal

Metabolism studies were carried out in two livestock species and in the rat:

For ruminants:	Lactating goats
For poultry:	Laying hens

Three goat metabolism studies were conducted with bentazone and its 6-OH- and 8-OH-metabolites, respectively, all of which were considered appropriate in the initial evaluation. Bentazone was rapidly excreted *via* urine, mainly as unchanged parent and small amounts of 6-OH- and 8-OH-bentazone. Feeding of 6-OH- and 8-OH-bentazone to goats revealed even faster excretion compared to parent bentazone. Only minor metabolites were detected in milk or edible tissues in all three studies. Metabolism studies with bentazone and its 6-OH- and 8-OH-metabolites in laying hens showed a similar metabolism pathway and excretion behaviour compared to goats and rats and were considered appropriate in the initial evaluation. Overall, goat and poultry studies showed minimal transfer of residues to meat, fat, edible organs, milk and eggs. No additional animal metabolism studies were necessary to estimate the fate of residues in livestock. Considering 6-OH-bentazone representing the main residue in feed items but being more rapidly excreted than bentazone it seems appropriate to include the partially conjugated hydroxy-metabolites in the residue definition for animal matrices.

6.11.1.3 Residues

In Table 6.11/3 a summary of the established bentazone EU maximum residue levels is given for food of plant and animal origin (source: Regulation (EC) No. 396/2005, last amended by Regulation (EU) No.893/2010). All existing MRLs are listed.

Table 6.11/3 Maximum residue levels for bentazone[#] in the EU

Crop/Tissue	t-EU MRL
1. FRUIT FRESH OR FROZEN; NUTS	0.1*
2. VEGETABLES FRESH OR FROZEN	-
(i) Root and tuber vegetables	0.1*
(ii) Bulb vegetables	0.1*
(iii) Fruiting vegetables	-
(a) Solanacea	0.1*
(b) Cucurbits - edible peel	0.1*
(c) Cucurbits-inedible peel	0.1*
(d) Sweet corn	0.3
(e) Other fruiting vegetables	0.1*
(iv) Brassica vegetables	0.1*
(v) Leaf vegetables & fresh herbs	-
(a) Lettuce and other salad plants including Brassicacea	0.1*
(b) Spinach & similar (leaves)	0.1*
(c) Vine leaves (grape leaves)	0.1*
(d) Water cress	0.1*
(e) Witloof	0.1*
(f) Herbs	10[1]
(vi) Legume vegetables (fresh)	-
Beans (with pods) (Green bean (french beans, snap beans), scarlet runner bean, slicing bean, yardlong beans)	0.3[1]
Beans (without pods) (Broad beans, Flageolets, jack bean, lima bean, cowpea)	0.05[1]
Peas (with pods) (Mangetout (sugar peas))	0.5
Peas (without pods) (Garden pea, green pea, chickpea)	0.2
Lentils	0.1*
Others	0.1*
(vii) Stem vegetables (fresh)	0.1*
(viii) Fungi	0.1*
(ix). Sea weeds	0.1*
3. PULSES, DRY	0.1*
4. OILSEEDS AND OILFRUITS	0.1*
5. CEREALS	0.1*
6. TEA, COFFEE, HERBAL INFUSIONS AND COCOA	0.1*
7. HOPS (dried) , including hop pellets and unconcentrated powder	0.1*
8. SPICES	0.1*
9. SUGAR PLANTS	0.1*
10. PRODUCTS OF ANIMAL ORIGIN-TERRESTRIAL ANIMALS	-
(i) Meat, preparations of meat, offals, blood, animal fats fresh chilled or frozen, salted, in brine, dried or smoked or processed as flours or meals other processed products such as sausages and food preparations based on these	0.05*
(ii) Milk and cream, not concentrated, nor containing added sugar or sweetening matter, butter and other fats derived from milk, cheese and curd	0.02*
(iii) Birds eggs, fresh preserved or cooked Shelled eggs and egg yolks fresh, dried, cooked by steaming or boiling in water, moulded, frozen or otherwise preserved whether or not containing added sugar or sweetening matter	0.05*

(*) indicates the lower limit of analytical determination
(#) sum of bentazone and the conjugates of 6-OH- and 8-OH-bentazone expressed as bentazone; for products of animal origin-terrestrial animals: bentazone
(1) EFSA Reasoned Opinion, EFSA Journal 2011;9(5):2188

Plants

Root and tuber vegetables

Potatoes

In 8 trials conducted in 2009 in Southern Europe, total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) at harvest (PHI F) ranged from <0.03-0.08 mg/kg after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha.
In the Annex II Dossier (1995), 13 trials (Northern Europe) on potatoes were presented with total residues at or below the LOQ of 0.06 mg/kg at DALA 28-140. The application rate was 1 x 0.96 or 1.2 kg a.s./ha.
Guidelines allow extrapolation from potatoes to tropical root vegetables. An EU MRL of 0.15 mg/kg was calculated for potatoes. Therefore, it is proposed to raise the established EU MRL of 0.1 mg/kg to **0.15 mg/kg** for **potatoes and tropical root vegetables**.

Bulb vegetables

Bulb onions

In 12 trials conducted in 1999-2009 in Northern (4 trials) and Southern Europe (8 trials), total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) at harvest (PHI F) were <0.06 mg/kg in Northern Europe and ranged from <0.03-0.04 mg/kg in Southern Europe. The trials in Northern Europe were performed with an exaggerated application rate of 1.55 kg a.s./ha compared to the current registered cGAP with an application rate of 0.96 kg a.s./ha.
Guidelines allow extrapolation from bulb onions to garlic and shallots. An EU MRL of 0.06 mg/kg was calculated for bulb onions. However, it is proposed to maintain the established EU MRLs of **0.1 mg/kg** for **onions, garlic and shallots**.

Spring onions

In 4 trials conducted in 2009 in Northern (2 trials) and Southern Europe (2 trials), total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) at harvest (PHI F) ranged from <0.03-0.06 mg/kg in Northern Europe and were <0.03 mg/kg in Southern Europe after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha.
Guidelines allow extrapolating from spring onions to Welsh onions and chives as well as to leek. Therefore, it is proposed to maintain the established EU MRLs of **0.1 mg/kg** for **spring onions, Welsh onions and chives and for leek**.

Fruiting vegetables

Sweet corn

In 2 trials conducted in 2007 in Northern (1 trial) and Southern Europe (1 trial), total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) at PHI 28±1 days were <0.03 mg/kg in Northern and 0.06 mg/kg in Southern Europe after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha.
Guidelines allow extrapolation from immature maize to sweet corn. Total bentazone residues in immature maize cobs without husks at PHI 28±1 days ranged from <0.03-0.20 mg/kg in Northern Europe (5 trials) and from <0.03-0.10 mg/kg in Southern Europe (5 trials) after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha.
An EU MRL of 0.4 mg/kg was calculated for **sweet corn**. Therefore, it is proposed to raise the established EU MRL of 0.3 mg/kg to **0.4 mg/kg**.

Fresh herbs

Thyme

In 4 trials conducted in 2005-2006 in Southern Europe, total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) at harvest (PHI F) ranged from <0.05-0.087 mg/kg after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha. Since thyme is an herb used predominantly in the Mediterranean cuisine, the Southern European data is considered appropriate for MRL calculation.
An EU MRL of 0.15 mg/kg was calculated. However, it is proposed to maintain the EU MRL of **10 mg/kg** for **thyme** as recommended by EFSA in the Reasoned Opinion on the modification of the existing MRLs for bentazone in legume vegetables and fresh herbs (EFSA Journal 2011;9(5):2188).

Legume vegetables

Beans and peas with pods

Guidelines allow extrapolation from beans with pods to peas with pods.
In 16 trials on beans conducted in 2008-2009 in Northern (7 trials) and Southern Europe (9 trials), total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) at PHI 28±1 days (or later if higher residues occurred) ranged from 0.03-0.10 mg/kg in Northern Europe and from <0.03-0.21 mg/kg in Southern Europe after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha.
In 2 trials on peas conducted in 2007 in Northern Europe, total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) at PHI 28±1 days were 0.04-0.05 mg/kg after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha.
An MRL of 0.3 mg/kg was calculated for beans with pods. Therefore, it is proposed to adjust the established EU MRL of 0.1 mg/kg to **0.3 mg/kg** for **beans with pods** as recommended by EFSA in the Reasoned Opinion on the modification of the existing MRLs for bentazone in legume vegetables and fresh herbs (EFSA Journal 2011;9(5):2188).
For peas with pods, it is proposed to maintain the established EU MRL of **0.5 mg/kg** for **peas with pods** as recommended by EFSA in the Reasoned Opinion on the modification of the existing MRLs for bentazone in legume vegetables and fresh herbs (EFSA Journal 2011;9(5):2188).

Beans and peas without pods

In the EFSA Reasoned Opinion (EFSA Journal 2011;9(5):2188), it was extrapolated from beans without pods to peas without pods.
In 15 trials on beans conducted in 2008-2009 in Northern (6 trials) and Southern Europe (9 trials), total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) at PHI 28±1 days (or later if higher residues occurred) were <0.03-0.03 mg/kg in Northern Europe and ranged from <0.03-0.04 mg/kg in Southern Europe after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha.
In 2 trials on peas conducted in 2007 in Northern Europe, total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) at PHI 28±1 days (or later if higher residues occurred) were <0.03 mg/kg after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha.
An EU MRL of 0.05 mg/kg was calculated for beans without pods and recommended by EFSA in the Reasoned Opinion on the modification of the existing MRLs for bentazone in legume vegetables and fresh herbs (EFSA Journal 2011;9(5):2188). Despite this, an MRL of **0.06 mg/kg** is proposed for **beans without pods** since it is not reasonable to set an MRL below the default MRL, which is proposed as 0.06 mg/kg in this document.
For peas without pods, it is proposed to maintain the established EU MRL of **0.2 mg/kg** for **peas without pods** as recommended by EFSA in the Reasoned Opinion on the modification of the existing MRLs for bentazone in legume vegetables and fresh herbs (EFSA Journal 2011;9(5):2188).

Pulses (dry)

Dried beans

In 15 trials conducted in 1999-2010 in Northern (5 trials) and Southern Europe (10 trials), total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) at harvest (PHI F) ranged from <0.06-0.06 mg/kg in Northern Europe and from <0.03-0.06 mg/kg in Southern Europe.
Overdosed trials (1.536-1.67 kg a.s./ha) in Northern (5 trials) and Southern Europe (8 trials) compared to the current registered cGAP with an application rate of 0.96 kg a.s./ha were included as total bentazone residues were at or below the LOQ of 0.06 mg/kg.
Guidelines allow extrapolation from green beans with pods to the whole group of pulses (dry) if the application of the active substance is performed before the consumable part of the crop has started to form. An EU MRL of 0.1 mg/kg was calculated for dried beans, while for green beans with pods, an MRL of **0.3 mg/kg** has been recommended by EFSA in the Reasoned Opinion on the modification of the existing MRLs for bentazone in legume vegetables and fresh herbs (EFSA Journal 2011;9(5):2188) (see above). Therefore, it is proposed to adjust the established EU MRL of 0.1 mg/kg for the **whole group of pulses (dry)** to 0.3 mg/kg.

Dried peas

Guidelines allow extrapolation from green beans with pods to the whole group of pulses (dry) if the application of the active substance is performed before the consumable part of the crop has started to form. Therefore, it is proposed to adjust the established EU MRL of 0.1 mg/kg for the whole group of pulses (dry) to 0.3 mg/kg (see above).

Oilseeds

Soybean

In 15 trials conducted in 2007-2009 in Northern (3 trials) and Southern Europe (12 trials), total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) at harvest (PHI F) were <0.03 mg/kg after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha.
Since all residues were below the LOQ, the number of residue trials is considered to be sufficient.
An EU MRL of 0.03 mg/kg was determined for soybean.

Linseed

In 4 trials conducted in 1999 in Southern Europe, total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) at harvest (PHI F) were <0.06 mg/kg. All trials were overdosed (about 1.5 kg a.s./ha). Even after application of this exaggerated rate compared to the current registered cGAP with an application rate of 0.96 kg a.s./ha, residues were below the LOQ. If all residues are below the LOQ, two residue trials per region are sufficient. Therefore, the four trials performed in Southern Europe are assumed to cover the Northern European region as well.
An EU MRL of 0.06 mg/kg was determined for linseed.

Guidelines allow extrapolation from soybean to the whole group of oilseeds except peanuts. To cover the calculated MRL values of both soybean and linseed, it is proposed to maintain the established EU MRL of **0.1 mg/kg** for the **whole group of oilseeds except peanuts**.

Cereals

Maize

In 28 trials conducted in 1998-2010 in Northern (12 trials) and Southern Europe (16 trials), total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) at harvest (PHI F) ranged from <0.03-<0.06 mg/kg. Results from exaggerated trials (1 x 1.55 kg a.s./ha compared to the current registered cGAP with an application rate of 0.96 kg a.s./ha) are also included (2 N-EU, 8 S-EU); the residues were below the LOQ of 0.06 mg/kg.
Guidelines allow extrapolation from maize to millet and sorghum. An EU MRL of 0.1 mg/kg was calculated for maize. Therefore, it is proposed to maintain the established EU MRLs of **0.1 mg/kg for maize, millet and sorghum**.

Wheat and barley

Guidelines allow extrapolation from barley, oats, rye, triticale or wheat to the remaining four crops. Therefore, wheat and barley residue trials are combined to determine a group MRL for cereals.
In 5 trials on barley conducted in 1999 in Southern Europe, total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) at harvest (PHI F) were <0.06 mg/kg. Results from exaggerated trials (1 x 1.55 kg a.s./ha compared to the current registered cGAP with an application rate of 0.96 kg a.s./ha) were used.
In 11 trials on wheat conducted in 1998 and 2010 in Northern (8 trials) and Southern Europe (3 trials), total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) at harvest (PHI F) were <0.03 mg/kg in Northern Europe and <0.06 mg/kg in Southern Europe. Results from exaggerated trials (1 x 1.55 kg a.s./ha compared to the current registered cGAP with an application rate of 0.96 kg a.s./ha) were used in Southern Europe.
An EU MRL of 0.06 mg/kg was determined for wheat and barley. However, it is proposed to maintain the established EU MRL of **0.1 mg/kg** for **barley, oats, rye, triticale and wheat**.

Herbal infusions (dried)

Valerian root

In 4 trials conducted in 2001-2003 in Northern Europe, total residues of the parent compound bentazone, 6-OH- and 8-OH-bentazone (in mg/kg bentazone) at harvest (PHI F) were <0.13 mg/kg after application according to the current registered cGAP±25% with an application rate of 0.96 kg a.s./ha.

Since the use in valerian roots is only intended in Northern Europe, the number of trials performed is sufficient for MRL determination.

Guidelines allow extrapolation from any single cultivated crop of herbal roots to roots of herbal infusions and spices. An EU MRL of **0.15 mg/kg** was determined for valerian roots. Therefore, it is proposed to adjust the established EU MRL of 0.1 mg/kg for **roots of herbal infusions and spices** to 0.15 mg/kg.

Livestock

In the three goat metabolism studies of bentazone, 6-OH- and 8-OH-bentazone which can serve as feeding studies in this case (see chapter 6.4), the nominal dose levels were 2 to 3 mg/kg bw/day. Thus, overdosing factors of 107, 5 and 188 (bentazone, 6-OH- and 8-OH-bentazone, respectively), based on the dietary burden for beef cattle as a worst case, have to be applied to the residues. All three metabolism studies included one further dose group which was highly overdosed and is therefore not used for calculations.
When samples of the three goat metabolism studies were analysed, overall residues in milk were 0.021-0.027 mg/kg. Application of the overdosing factors leads to an anticipated overall residue for total bentazone (sum of bentazone, 6-OH- and 8-OH-bentazone) of 0.004 mg/kg in milk.
In tissues, residues of 0.018-0.03, 0.118-0.6, 0.011-0.013 and 0.007-1.57 mg/kg were found in liver, kidney, muscle and fat for the three analytes, respectively. Application of the overdosing factors leads to anticipated residues of 0.003, 0.03, 0.002 and 0.02 mg/kg for total bentazone (sum of bentazone, 6-OH- and 8-OH-bentazone), respectively. Since feed burden calculations are conducted under various worst-case assumptions extrapolations give a very conservative estimate of the residues.

In the hen metabolism study, which can serve as a feeding study in this case (see chapter 6.4), the nominal dose level for total bentazone (bentazone, 6-OH- and 8-OH-bentazone) was 10 mg/kg bw/day. Thus, the overdosing factor of 500 (10 mg/kg bw/day / 0.02 mg/kg bw/day) has to be applied to the residues
When samples were analysed for total bentazone, residues in eggs were 0.20 mg/kg. Application of the overdosing factor leads to an anticipated residue of 0.0004 mg/kg in eggs.
In tissues, residues of 0.15, 0.06, 0.48, 0.41 and 1.46 mg/kg were found in subcutaneous fat, peritoneal fat, leg muscle, breast muscle and live, respectively. Application of the overdosing factor leads to anticipated residues of 0.0003, 0.0001, 0.001, 0.0008 and 0.0029 mg/kg, respectively.

Since the residue definition is proposed to be

> **Bentazone (sum of bentazone and the conjugates of 6-OH-bentazone and 8-OH-bentazone expressed as bentazone)**

and an overall LOQ of 0.03 mg/kg is supported for the sum of bentazone and the conjugates of 6-OH-bentazone and 8-OH-bentazone expressed as bentazone the default MRL should apply for all animal tissues, eggs and milk.

In conclusion, it is proposed to establish EU MRLs for **animal tissues, eggs and milk** of **0.03 mg/kg**.

6.11.1.4 Consumer safety

The EFSA PRIMo (rev. 2) was used to assess the chronic and the acute dietary exposure. Assessments were made based on the currently established EU MRLs or MRLs proposed in the EFSA Reasoned Opinion 2011 and all newly proposed EU MRLs.

TMDI calculations led to an ADI utilisation of 4.2% for the WHO Cluster diet B, with wheat and herbs contributing 0.9% and 0.5% to the ADI, respectively. ADI utilisation for UK Toddler was 4.2% with sugar plants and milk and cream contributing 1.4% and 0.6% to the ADI, respectively.

The acute dietary risk assessment performed clearly indicates that there is no acute risk for any subpopulation group under consideration, the highest ARfD utilisation being 22.9%.

According to the calculations, the intake of bentazone (sum of bentazone, 6-OH- and 8-OH-bentazone) residues is unlikely to present a public health concern, even when the worst case situation is considered.

6.11.2 Reasonable grounds in support of the petition

The data available for bentazone address all data requirements in the area of metabolism, residues and consumer risk assessment. Adequate data exist to establish MRLs in all relevant target crops and calculated intakes show that no risks to consumers will occur from the application of products containing bentazone according to GAP.

BASF
The Chemical Company

DOSSIER FOR THE EVALUATION OF THE ACTIVE SUBSTANCE

Bentazone (BAS 351 H)

Data requirement according to Regulation (EU) No. 544/2011 and (EU) No. 545/2011 (formerly Annex II and III of Directive 91/414/EEC) using OECD Guidance for Industry Data Submissions on Plant Protection Products and their Active Substance (Dossier Guidance)

Document M-II

Summary and evaluation (Tier II)

Section 5

Fate and behaviour in the environment

BASF DocID 2012/1007335

compiled by

[redacted]

Date: 24 February 2012

7 Fate and Behaviour in the Environment

List of Contents

According to Article 1(c) of Regulation (EU) No. 1141/2010 the supplementary dossier includes data and risk assessments which were not part of the original dossier and which are necessary to reflect changes. Reasoning for each test or study as required by Art 1(d)/(e) of the Regulation is given in the reference list of the dossier.

For Bentazone a literature search has been performed. The search report including a description of the selection and assessment process is provided in BASF DocID 2012/1007281 (DocLIIA).
The results of the selection process and assessments for Fate and behaviour in the environment are outlined in BASF DocID 2012/1007285.

7.1 Route of degradation in soil - laboratory studies

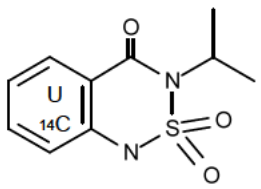
Bentazone (BAS 351 H), a selective herbicide for use in cereals, maize, legumes and various other crops, is registered in Europe since many years. It was fully reviewed under Directive 91/414/EEC and included on Annex I on 23. October 2000 (Commission Directive 2000/68/EC, with prolongation by Directive 2010/77/EU).

All relevant information on the first Annex I review including the endpoints used in environmental risk assessments can be found in the EU review report of bentazone 87585/VI/97 final as well as in the bentazone monograph 5737/ECCO/PSD/98 (DAR-predecessor).

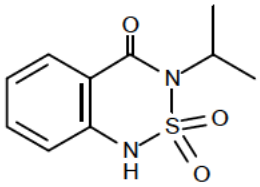
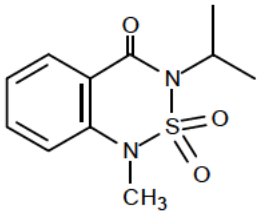
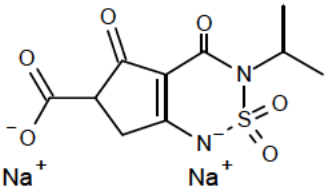
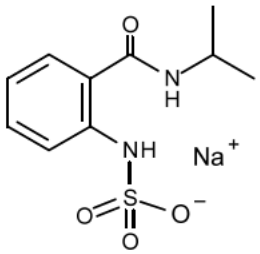
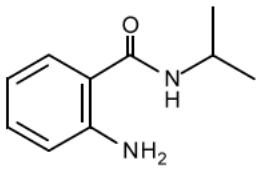
For the current Annex I renewal, a data gap analysis according to new guidelines, new guidance documents and new procedures in kinetic evaluations and exposure assessments was performed, and new studies / evaluations were initiated where considered necessary. In order to provide a comprehensive overview on existing environmental data on bentazone for Annex I renewal, each chapter of this section consists of a summary on the former EU agreed endpoints followed by summaries of the new studies and kinetic evaluations according to the current guidance required in the EU process.

Furthermore, a literature search was performed, and scientific publications were included into the dossier when considered endpoint relevant and being of sufficient high quality. All bentazone related publications on environmental fate found during the literature search were categorized according to (1) being endpoint relevant and of adequate quality; then summaries are provided in the appropriate dossier chapters, (2) potentially endpoint relevant, but showing no new information or having deficiencies, (3) having no endpoint relevance.

The already peer-reviewed studies as well as the new studies were performed using uniformly phenyl-ring ^{14}C-labelled bentazone.



A concordance list of structures and designations of reference compounds used during environmental fate studies is given below.

Compound designation	Reference code (Reg. No.)	Molecular weight (g/mol)	Structure
Bentazon BAS 351 H M351H000	51929	240.3	
N-methyl-bentazon BH 351-N-me. M351H009	79520	254.3	
"Peak B" M351H023	5819746 (5831080 = disodium-salt)	332.25	
"Peak C" M351H024	268168 (5051517 = sodium-salt)	280.28	
BH 351-AIPAM "AIPAM" M351H025	36848	178.23	

7.1.1 Aerobic degradation

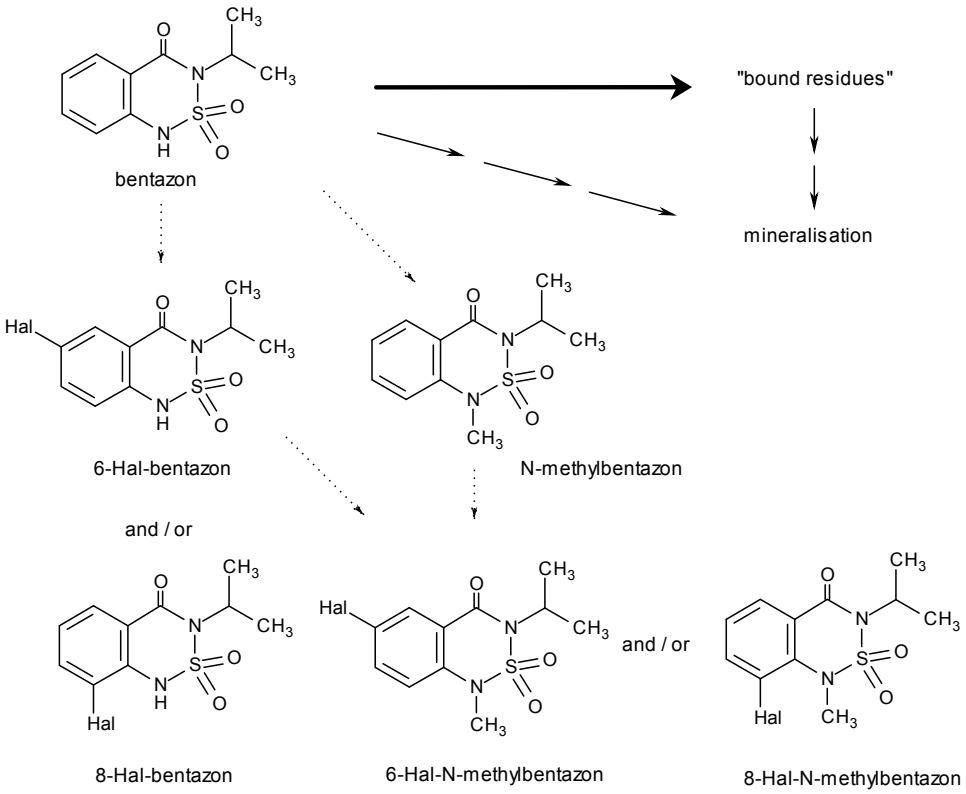
A brief summary of the aerobic degradation of bentazone in soil based on data considered within the former EU review process is presented below.

Bentazone degradation in soil is mainly characterized by incorporation into the organic soil structure with tight binding to fulvic acids, humic acids and humines. The initial attack of bentazone in soil is assumed to be hydroxylation at the phenyl ring in the 6- or 8-position. The direct detection of 6-OH and 8-OH-bentazone in soil is difficult because both phenol-like structures are assumed to instantly react with the organic soil matrix, forming unextractable residues. Final degradation will then be slow mineralization to CO_2.

Minor pathways were the methylation and halogenation (prevailing chlorination, but also bromination) of the active ingredient. Both reactions are known to be performed by soil micro-organisms. All those metabolites occurred only in minor amounts in soil. Since N-methyl-bentazone was the only metabolite which slightly exceeded 5% of applied radioactivity in two consecutive soil samplings, it is now considered for further environmental risk assessments according to new guidance documents introduced since last Annex I inclusion.

In old studies, soil photolysis was shown to be no significant degradation route of bentazone with an almost unchanged concentration of bentazone in soil after about 39 days exposure. A new soil photolysis study however showed a slightly increased degradation rate under irradiated conditions, leading to an enhanced incorporation into humic substances. Metabolites remained < 5% of applied radioactivity.

Proposed pathway for the degradation of bentazone in soil (as evaluated already during former Annex I inclusion)



This proposed pathway is still considered valid. Nevertheless, since the existing aerobic soil metabolism study was rather old (1987) and not performed according to a valid soil metabolism guideline and was overdosed (appl. rate of 7.5 kg/ha compared to the maximum recommended application rate of 1.0 kg/ha), a new aerobic soil metabolism study with ^{14}C-labelled bentazone was initiated. This study was performed to fulfil formal data requirements. No new results on soil behaviour were expected.

Report:	II A 7.1.1/1 Staudenmaier H., Kuhnke G. 2010(b) Aerobic soil metabolism of ^{14}C-Bentazone BASF DocID 2010/1057318
Guidelines:	OECD 307 (2002); BBA IV 4-1; EPA 162-1; SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995); EPA 835.4100
Testing Laboratory and dates:	BASF SE; Limburgerhof; Germany Fed.Rep. 22-Jun-2009 - 23-Mar-2010
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The aerobic soil metabolism of bentazone was investigated in a sandy loam (Bruch West, Limburgerhof, Germany, classified according to USDA) freshly collected from the field and passed through a 2 mm sieve before use. The soil was treated with [^{14}C-phenyl]-bentazone at a nominal rate of 2.7 mg per kg dry soil which corresponds to a field application rate of 1000 g bentazone per hectare, calculated on the basis of an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g/cm³.

Soil aliquots of 100 g (dry weight basis) were weighed into test vessels and incubated in the dark under aerobic conditions at soil moisture of 40% of the maximum water holding capacity and a temperature of 20°C. A closed incubation system with continuous aeration (moistened air) was used with an attached trapping system for the collection of volatile compounds. At two time points during incubation (57 and at 126 DAT), the microbial biomass was determined by the substrate induced respiration method, verifying that the soil was viable throughout the incubation period.

Samples were taken at 0, 1, 3, 7, 14, 30, 64, 91, 120 and 150 days after treatment (DAT). At sampling times 0, 30, 120 and 150 DAT, soil samples were worked up in duplicate.

The soil samples were extracted twice with methanol and twice with water/methanol (v:v, 1:1). The amount of radioactivity in the individual extracts was determined by liquid scintillation counting (LSC). The methanol and water/methanol extracts per soil sample were combined, respectively, and analysed by means of HPLC. The remaining soil was homogenized and combusted after extraction to determine the amount of non-extractable residues (NER) in soil. The NER were further characterized by NaOH extraction and subsequent fractionation into fulvic acids, humic acids and humins. The fulvic acid fraction was furthermore partitioned with ethyl acetate. A full material balance was provided for each sampling interval.

The results showed that the amount of extractable radioactivity in soil continuously decreased from 102.2% of the total applied radioactivity (TAR) at 0 DAT to 6.9% TAR after 150 days of incubation.

The amount of the test item bentazone decreased from 101.0% TAR at 0 DAT to 2.3% TAR at 150 DAT. Metabolites were formed only in minor amounts of which the most prominent metabolite (max. 2.8% TAR) was identified as N-methyl-bentazone (M351H009). All other metabolites were formed in even lower amounts and their sum in the total extracts never exceeded 2.2% TAR at any sampling time.

Mineralization to ^{14}C-CO_2 reached a total of 9.0% TAR after 150 days of incubation. No other volatile compounds were detected.

Non-extractable radioactive residues (NER) were formed in considerable amounts during incubation. They increased from 3.3% TAR on day 0 to a maximum of 68.8% TAR after 150 days. After extraction with NaOH, still about half of the radioactivity (2.3 – 34.8% TAR) remained tightly bound to the soil matrix (humines). The NaOH extractable radioactivity was distributed between the humic acid (1.0 – 11.9% TAR) and the fulvic acid fraction (2.7 – 21.0% TAR) in a ratio of about 1:2. The fulvic acid fraction was further characterized by partitioning with ethyl acetate. Amounts of 1.6 to 7.3% TAR of the fulvic acid fraction were soluble in ethyl acetate, whereas 0.9 to 13.1%TAR remained in the water phase. The ethyl acetate soluble fractions from samples of 30 DAT to 120 DAT were investigated by HPLC. Parent compound was found to be the most prominent peak, accounting for 3.0 to 3.7% TAR.

The material balance throughout the incubation period ranged from 92.6 to 105.5% TAR except for the 150 DAT sampling, for which a material balance of only 84.7 TAR was achieved. The average material balance for all soil samples was 98.0% TAR.

Kinetic analysis and calculation of DT_{50} and DT_{90} values for bentazone was performed following the recommendations of the FOCUS Kinetics workgroup. The analysis was done by non-linear regression methods using the software package KinGUI version 1.1.

The best-fit DT_{50} value was calculated to be 45.1 days (SFO kinetics), the corresponding DT_{90} value was 150 d.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

BAS code:	BAS 351 H
Reg.No.:	51929
CAS-No.:	25057-89-0
Chemical name (IUPAC):	3-isopropyl-1H-2,1,3,-benzothiadiazin-4(3H)-one-2,2-dioxide
Molecular weight:	240.28 g/mol (unlabelled)
Position of radiolabel:	phenyl-U-^{14}C
Batch-No.:	210-2301
Specific radioactivity:	5.15 MBq/mg (309000 dpm/µg)
Radiochemical purity:	96.5%

2. Soil

A sandy loam (according to USDA) was used for the aerobic soil metabolism. The soil was freshly taken from the field, passed through a 2 mm sieve, remoistened to approximately 8-12% soil moisture and stored for no longer than 3 months at 4°C before use. Seven days before treatment, the soil moisture was adjusted to 40% of the maximum water holding capacity and the soil pre-incubated at room temperature to re-establish microbial activity. A summary of the soil characteristics is given in Table 7.1/1.

Table 7.1/1 Properties of soil Bruch West used to investigate degradation of [phenyl-U-^{14}C]- bentazone under aerobic conditions

Soil designation	Bruch West (09/060/03)
Origin	Limburgerhof, Germany
DIN Particle size distribution [%] sand 0.063 – 2 mm silt 0.002 – 0.063 mm clay < 0.002 mm textural class	 61.5 27.2 11.2 loamy sand
USDA Particle size distribution [%] sand 0.050 – 2 mm silt 0.002 – 0.050 mm clay < 0.002 mm textural class	 63.8 24.9 11.2 sandy loam
Organic C [%] Organic matter [%] *	1.23 2.12
pH (H_2O) pH ($CaCl_2$)	7.9 7.3
Cation exchange capacity [$cmol^+$ / kg]	12.2
Maximum water holding capacity [g/100g dry soil]	23.6
Microbial biomass (before start of study) [mg C/100g dry soil]	37.0
Microbial biomass (after 57 days) [mg C/100g dry soil]	31.7**
Microbial biomass (after 126 days) [mg C/100g dry soil]	28.4**

* organic matter = organic carbon x 1.724
** determined at BASF test facility Limburgerhof

B. STUDY DESIGN

1. Experimental conditions

The soil was treated with the phenyl-labelled test item at a nominal concentration of 2.7 mg ^{14}C-bentazone per kg dry soil, which corresponds to a field application rate of 1000 g/ha, calculated on the basis of an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g/cm³.

For application, the calculated amount of treatment solution was applied to 2800 g dry soil equivalents. The soil was thoroughly mixed with a hand mixer. Soil aliquots of 100 g (dry weight basis) were filled into test vessels. The amount of total applied radioactivity (TAR) was determined by combustion of 0.9-1 g aliquots of a 0-Day sample and subsequent LSC measurement.

The test vessels were placed in a temperature-controlled chamber (incubation cabinet) and incubated in the dark for up to 150 days at a temperature of 20°C. Throughout the incubation period, the samples were continuously aerated with a slight stream of moistened and CO_2 free air. The water content of the soil samples was monitored throughout the incubation period by weighing representative vessels at each sampling time.

During incubation, the volatiles were trapped in a trapping system of three gas washing flasks containing about 50 ml 0.5 M NaOH, 0.5 M H_2SO_4 and ethylene glycole, respectively.

For determination of the microbial biomass during and at the end of incubation, additional soil samples (1050 g dry soil equivalents) were treated with 1.9 ml acetone. These soil samples were incubated under the same conditions (dark, 20°C, moistened air) as the treated soil.

2. Sampling

Sampling times were 0 (no sampling of volatiles), 1, 3, 7, 14, 30, 64, 91, 120 and 150 DAT.

At 0, 30, 120 and 150 DAT two replicates were worked up. At the other sampling times only one replicate (replicate 1) was worked up whereas the second one was stored in a freezer. Samples that were not worked up immediately after application were frozen until further processing. The trapping solutions for volatiles were sampled at each sampling time and replaced by new flasks filled with fresh solutions.

3. Analytical procedures

Soil portions of 100 g dry soil equivalents were extracted twice with 100 mL methanol and twice with 100 mL water/methanol (v:v, 1:1) for about 30 minutes on a laboratory shaker, respectively. After each extraction step, the sample was centrifuged and the supernatant filtered into a 100 mL volumetric flask. The extracts were made up to volume with methanol, and three aliquots were measured by LSC.

The two methanol and the two water /methanol extracts were combined, respectively, and then concentrated to dryness by rotary evaporation. The dry residue was re-dissolved in about 5 mL methanol, sonicated and transferred into a 5.0 mL volumetric flask, which was filled to the calibration mark with solvent. Aliquots of the concentrated extracts were centrifuged for clean-up. Afterwards the supernatants were analysed by HPLC to obtain the metabolite pattern. The procedural recoveries after the concentration of the extracts were checked by LSC. Recoveries were >90 % for all extracts. The recoveries were again checked after centrifugation which also resulted in recoveries > 90% for all extracts.

The extracted soil was dried in a drying oven at 70°C. After weighing, the soil was stored in a freezer until further processing. To determine the amount of non-extractable residues (NER) the soil was homogenized in a laboratory mill and aliquots thereof were combusted. The evolved ^{14}C-CO_2 from each combusted aliquot was trapped in Oxysolve C-400 scintillator and measured by LSC.

The solutions for the trapping of volatiles were collected at all sampling times except at day 0. They were made up to volume and aliquots were measured for radioactivity. If the measurement was not conducted immediately after sampling, samples were frozen until further processing.

Non-extractable radioactive residues (NER) were further characterized by NaOH extraction for samples taken from 1 DAT onwards (replicate 1). Dry and homogenized soil aliquots of 50 g were transferred into a centrifuge vessel and shaken for 13 h with 50 mL 0.5 M NaOH on a horizontal laboratory shaker. After centrifugation (about 14000 rpm), the supernatant was decanted into a 50 mL volumetric flask. The NaOH extraction was repeated twice (for 6 h and 19 h, respectively). The radioactivity of the extracts was determined by LSC.

The remaining soil samples were oven dried at 70°C. Afterwards samples were homogenized in a laboratory mill. Four aliquots of approximately 1 g were combusted and the resulting ^{14}C-CO_2 trapped measured by LSC in order to determine the ^{14}C-residues in the non-extractable humins.

To separate the fulvic and humic acids, the NaOH extracts were combined and transferred into a centrifuge tube. The NaOH extracts were adjusted to pH 1.5 ± 0.2 by adding concentrated HCl. After complete precipitation, the suspension was centrifuged. The centrifugate (fulvic acids) was filled in a graduated cylinder and the volume was determined. The precipitated humic acids were re-dissolved in 50 mL 0.5 M NaOH and the solution was transferred into a 100 mL volumetric flask. Both the humic acid and the fulvic acid fractions were analysed by LSC.

The fulvic acid fraction was further partitioned three times with 70 ml ethyl acetate, respectively. The ethyl acetate extracts were combined and measured by LSC as well as the remaining aqueous fraction.

For samples taken from 30 DAT onwards, the combined ethyl acetate phase was concentrated to dryness using a rotary evaporator. The remainder was transferred into a pear-shaped flask using about 10 ml MeOH and concentrated to dryness again. The dry residue of each sample was dissolved in 1.5 ml MeOH+water (80+20), sonicated and transferred into Eppendorf cups. After centrifugation for 2 min at 14000 rpm, two 25 µl aliquots were measured by LSC to determine the procedural recoveries which were 100-101%. An aliquot was analysed by HPLC.

The microbial biomass in the soil samples treated with solvent only was determined after 57 and 126 days of incubation. The method was based on the determination of the oxygen consumption upon addition of glucose using a “BSB digi” (Johanna Otto GmbH). For that purpose, 250 mg glucose was added to 100 g portions of soil (dry mass) each, and the amended soil incubated at 22°C for 6 hours in the closed system of the BSB digi. The oxygen consumed by the microbes was measured and replaced continuously by the system. The output from the system was converted to microbial biomass by the formula: 1 mg O_2/h = 28.4 mg C biomass. All biomass measurements were made in triplicate.

4. Kinetic modelling

The measured residues of the applied substance were analysed in order to determine the degradation kinetics and trigger endpoints for persistence (DT_{50}, DT_{90}) following the recommendations of the FOCUS Kinetics workgroup [*FOCUS (2006) "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434pp.*]; (non-GLP).

The analysis was conducted by non-linear regression methods employing the software tool KinGUI 1.2 [SCHÄFER, D., MIKOLASCH, M., RAINBIRD, P., HARVEY, B. (2007) KinGUI: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics. In: Del Re, A.A.M. et al. (Eds.): Proceedings of the XIII Symposium on Pesticide Chemistry, Piacenza, 2007, P. 916-923.].

For each data set, the kinetic models proposed by the FOCUS Kinetics guidance document were tested in order to identify the best-fit model. The recommended kinetic models, i.e. the single first order kinetics (SFO) and the Gustafson-Holden model (FOMC) are already implemented in KinGUI. The Goodness-of-fit was evaluated by visual assessment, χ^2 minimum error, and type-I-error rate (t-test) [for details see Chapter 6.3 in FOCUS (2006)].

In all soils, the measured value at DAT 0 was set to the material balance. At later sampling time points (1, 3, 7, 14, 30, 64, 91, 120 and 150 DAT), the measured values were set to the amount of extractable bentazone residues. When available, replicate measurements were used for parameter estimation.

II. RESULTS AND DISCUSSION

The results on distribution of radioactivity and material balance in soil treated with phenyl-labelled ^{14}C-bentazone are summarized in Table 7.1/2.

A. MASS BALANCE

The material balance throughout the incubation period ranged from 92.6 to 105.5% TAR except for the 150 DAT sampling, for which a material balance of only 84.7 % TAR was achieved. The average material balance for all soil samples was 98.0% TAR.

B. EXTRACTABLE AND BOUND RESIDUES

The amount of extractable radioactivity continuously decreased from 102.2% TAR at 0 DAT to 6.9% TAR at the end of the study after 150 days of incubation. The majority of the extractable radioactive residues was always obtained by extraction with methanol. The subsequent extraction with water/methanol (v:v, 1:1) yielded additional 2.3% to 15.0% TAR.

The amount of non-extractable radioactive residues (NER) increased during the course of the study from 3.3% TAR on day 0 to 68.8% TAR at the end of the study after 150 days.

Table 7.1/2 Distribution of radioactivity in soil Bruch West after treatment with [phenyl-U-^{14}C]-bentazone and aerobic incubation at 20°C [%TAR]

Days after treatment	Extractable			NER	Volatiles		Material balance
	Methanol	Methanol/ H_2O	Total		CO_2	Other volatiles*	
0	86.0	15.0	101.0	3.4	n.d.	n.d.	104.4
0	88.4	15.0	103.4	3.3	n.d.	n.d.	106.6
0 mean	87.2	15.0	102.2	3.3	n.d.	n.d.	105.5
1	84.8	13.4	98.2	5.8	0.1	0.0	104.1
3	85.9	11.0	96.9	8.8	0.3	0.0	106.0
7	81.2	10.6	91.8	11.4	0.7	0.0	103.9
14	73.6	10.1	83.8	15.3	1.2	0.0	100.3
30	60.7	11.0	71.6	24.6	2.3	0.0	98.6
30	61.5	10.7	72.2	25.1	2.3	0.0	99.6
30 mean	61.1	10.8	71.9	24.9	2.3	0.0	99.1
64	37.6	8.6	46.1	49.0	4.4	0.0	99.5
91	24.4	6.1	30.5	56.9	6.5	0.0	93.9
120	15.4	4.7	20.1	64.7	8.2	0.0	93.0
120	15.3	4.7	20.0	63.9	8.2	0.0	92.2
120 mean	15.3	4.7	20.1	64.3	8.2	0.0	92.6
150	4.3	2.2	6.5	66.9	9.0	0.0	82.4
150	4.9	2.3	7.2	70.7	9.0	0.0	86.9
150 mean	4.6	2.3	6.9	68.8	9.0	0.0	84.7

* = sum of radioactivity in H_2SO_4 and ethylene glycol traps
n.d. = not determined
NER = non-extractable residues
TAR = total applied radioactivity (100% = 2.6839 mg/kg dry soil)

C. VOLATILIZATION

Soil samples treated with ^{14}C-bentazone showed moderate mineralization, with CO_2 amounts reaching a total of 9.0% TAR after 150 days of incubation. No other volatile compounds were observed.

D. TRANSFORMATION OF PARENT COMPOUND

The results of the HPLC analysis are shown in Table 7.1/3.

Throughout the incubation period the most prominent peak present in the extracts consisted of the parent compound bentazone. During the course of the study, its amount decreased from 101.0% TAR at 0 DAT to 2.3% TAR after 150 days of incubation. The identification of the parent compound was achieved by chromatographic comparison with the retention time of the ^{14}C-test item and the structure was additionally confirmed by means of mass spectrometry.

Besides parent compound one further peak with a retention time of 44.9 min could be identified. Mass spectrometry analysis showed that this compound consisted of N-methyl-bentazone (M351H009). It was found from 30 DAT onwards and increased slightly to a maximum of 2.8% TAR at 120 DAT. At end of incubation it was detected with about 2.4% TAR.

Besides the parent compound and N-methyl-bentazone, a number of unknown metabolites were present in the soil extracts. They were formed in low amounts only and their sum in the total extracts never exceeded 2.2% TAR. They are summarized as "sum others" in the results table.

Table 7.1/3 Radio-HPLC-analysis of soil extracts after treatment of soil Bruch West with [phenyl-U-^{14}C]-bentazone and aerobic incubation at 20°C [%TAR]

days after treatment	^{14}C total extractable	Bentazone (BAS 351 H) t_R ~35.8'	N-methyl-bentazone (M351H009) t_R~44.9'	Sum others
0	101.0	99.7	n.d.	1.2
0	103.4	102.2	n.d.	1.2
0 mean	102.2	101.0	n.d.	1.2
1	98.2	97.2	n.d.	1.0
3	96.9	95.8	n.d.	1.1
7	91.8	91.0	n.d.	0.8
14	83.8	83.0	n.d.	0.8
30	71.6	70.1	0.6	0.9
30	72.2	70.6	0.6	1.0
30 mean	71.9	70.4	0.6	0.9
64	46.1	43.2	1.7	1.3
91	30.5	26.6	2.2	1.7
120	20.1	15.4	2.7	2.0
120	20.0	15.0	2.9	2.1
120 mean	20.1	15.2	2.8	2.0
150	6.5	2.1	2.4	2.0
150	7.2	2.4	2.3	2.4
150 mean	6.9	2.3	2.4	2.2

t_R = retention time [min]

Characterization of non-extractable residues

From 1 DAT to 120 DAT, the non-extractable radioactive residues were further characterized by NaOH extraction and subsequent fractionation into fulvic acids, humic acids and humins. The results are shown in Table 7.1/4 (in % TAR).

After extraction with NaOH, still about half of the radioactivity (2.3 – 34.8% TAR) remained tightly bound to the soil matrix (humines). The NaOH extractable radioactivity was distributed in a ratio of about 1:2 between humic acids (1.0 – 11.9% TAR) and fulvic acids (2.7 – 21.0% TAR).

The fulvic acid fraction was further partitioned with ethyl acetate. Amounts of 1.6 - 7.3% TAR were detected in the ethyl acetate phase and amounts of 0.9 - 13.1 % TAR were found in the aqueous phase. The ethyl acetate phases from sampling times 30 to 120 DAT were analysed by HPLC. Parent compound accounted for only 3.0 to 3.7% TAR. The amount of a.i. increased slightly towards 91 DAT and decreased further on. Additionally, numerous breakdown products were detected in small amounts.

Table 7.1/4 Characterization of non-extractable residues in soil Bruch West after treatment with [phenyl-U-^{14}C]-bentazone and aerobic incubation at 20°C [%TAR]

days after treatment	NER	NaOH extract	Fulvic acids			Humic acids	Humins
			total	Ethyl acetate soluble	Acidic water soluble		
1	5.8	3.6	2.7	1.6	0.9	1.0	2.3
3	8.8	5.0	3.4	1.9	1.4	1.5	3.8
7	11.4	6.4	4.3	2.3	1.8	1.9	5.1
14	15.3	8.3	5.7	3.0	2.5	2.5	7.1
30*	24.6	12.9	8.5	4.2	4.1	4.0	12.7
64	49.0	26.0	16.6	6.8	9.1	8.8	23.6
91	56.9	31.2	19.3	6.6	12.1	10.8	30.4
120*	64.7	34.1	21.0	7.3	13.1	11.9	34.8

* replicate 1
n.d. = not determined
NER = non-extractable residues
TAR = total applied radioactivity (100% = 2.6839 mg/kg dry soil)

The high portions of radioactivity in the high molecular humic acids and unextractable humins confirm the overall knowledge about bentazone that it is tightly incorporated into the humic substances and cannot be released even with harsh extraction solvents like 0.5 N NaOH.

E. KINETIC MODELLING RESULTS

The estimated DT_{50} and DT_{90} values of bentazone in aerobic soil are shown in Table 7.1/5.

Table 7.1/5 Estimated DT_{50} and DT_{90} values and χ^2 - error level

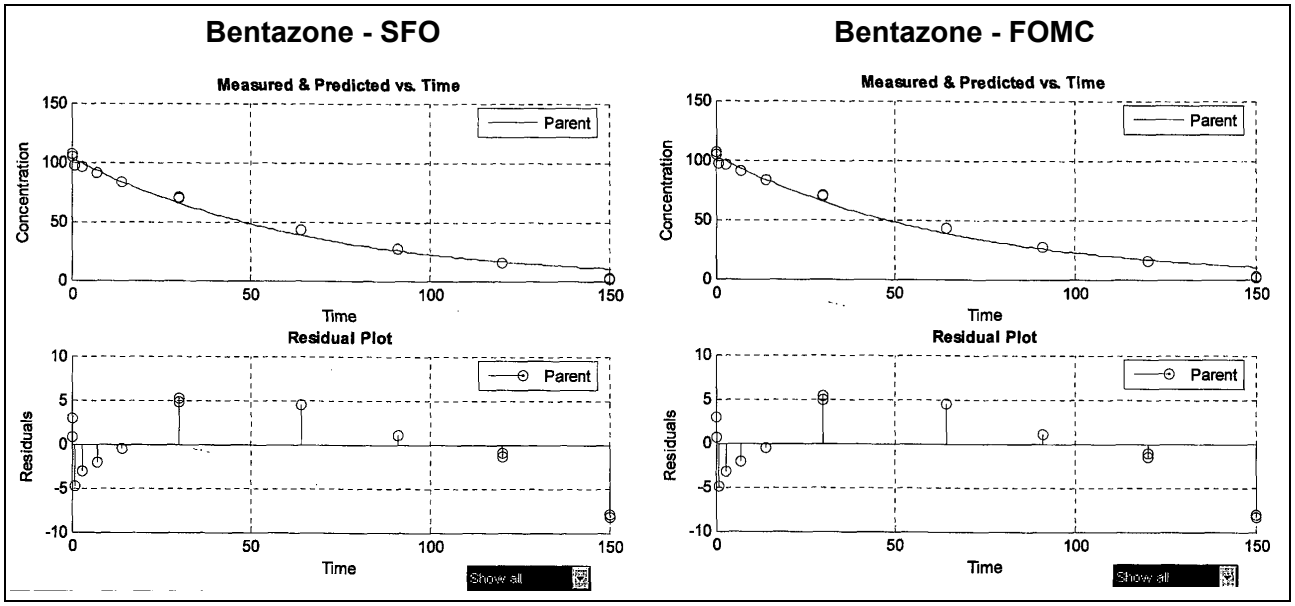
Kinetic model	Substance	DT_{50} [d]	DT_{90} [d]	χ^2 - error level [%]	Visual fit
SFO	Bentazone	45.1	149.8	5.4*	Good
FOMC	Bentazone	44.8	150.6	5.7	Good

*: best fit model

The corresponding plots of modelled against observed residues are presented in Figure 7.1/1.

The SFO model was more appropriate than the FOMC model, providing an excellent fit and a lower χ^2 error.

Figure 7.1/1 ^{14}C-phenyl-labeled bentazone: modelled against observed residue



III. CONCLUSION

From the results of the present study, it is concluded that bentazone degrades relatively fast in soil Bruch West when incubated under aerobic conditions at a soil moisture of 40% of the maximum water holding capacity and a temperature of 20°C.

Metabolites were formed only in very low amounts. Even the most prominent metabolite N-methyl-bentazone (M351H009) never exceeded 2.8% TAR.

Characterization of the non-extractable radioactive residues (NER) revealed that about half of the radioactivity was tightly bound to humins, while the rest was distributed in a ratio of about 1:2 between the humic acid and the fulvic acid fractions. Only by harsh extraction methods leading to destruction of the humic structure and subsequent analysis of the fulvic acids, a maximum of 3-4% of the overall 65% TAR bound residues could still assigned to bentazone.

CO_2 was formed in low amounts reaching 9% TAR at the end of the study after 150 DAT. No other volatile compounds were detected.

The SFO DT_{50} and DT_{90} values were 45.1 days and 149.8 days, respectively. These values are appropriate for use as persistence endpoints as well as modelling endpoints after normalisation to reference moisture conditions.

7.1.2 Anaerobic degradation

During the former Annex I listing process, no anaerobic soil metabolism study was submitted. Therefore, a new study was performed.

Report:	II A 7.1.2/1 Ebert D. 2010(b) Anaerobic soil metabolism of Bentazon (BAS 351 H) BASF DocID 2010/1005602
Guidelines:	OECD 307; BBA IV 4-1; EPA 162-2; EPA 835.4200
Testing Laboratory and dates:	BASF SE; Limburgerhof; Germany Fed.Rep. 28-May-2009 - 20-Jan-2010
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The anaerobic soil metabolism of ^{14}C-bentazone (BAS 351 H) was investigated in a German soil (Bruch West, Limburgerhof) collected freshly from the field and passed through a 2 mm sieve before use. It was treated at a concentration of about 2.4 mg bentazone/kg dry soil, which corresponds to a field application rate of about 900 g/ha when assuming an equal distribution in the upper 2.5 cm soil layer and a soil density of 1.5 g/mL. For the aerobic pre-incubation phase, the soil was adjusted to 40% of the maximum water holding capacity.

Treated soil portions of 100 g (dry weight basis) were weighed into glass test vessels and incubated for the first 14 days under aerobic conditions in the dark at a temperature of 20 ± 1°C. Then the half-life of bentazone in soil was almost reached and the soil was flooded with water (day 15). The aeration was switched to nitrogen in order to establish anaerobic conditions for the remaining incubation period. All exiting air/nitrogen was passed through a trapping system consisting of flasks containing ethylene glycole and aqueous sodium hydroxide for trapping organic volatiles and $^{14}CO_2$, respectively.

Samples were taken at 0, 3, 7, 14, 21, 28, 42, 77 and 120 days after treatment (DAT).

Soil samples were extracted with methanol and methanol/water mixtures. The extracts were measured for radioactivity by liquid scintillation counting (LSC), then combined, concentrated and subjected to radio-HPLC analysis. The non-extractable residues were determined by combustion and subsequent LSC measurement. The radioactivity in the volatile trapping solutions was also determined by LSC. A total balance of radioactivity was established for each sampling interval.

During the aerobic incubation phase, the amount of extractable radioactivity decreased rapidly from 100 %TAR at day 0 to 57 %TAR at day 14. After establishing anaerobic conditions from day 15 onwards, no significant further decline of extractability was observed. The extractable residues remained in the range of 53 – 57 %TAR until 120 days. The non-extractable ^{14}C-residues increased to 39 %TAR within the first 14 days under aerobic incubation and then remained at a rather constant level of 40 - 43 %TAR under anaerobic conditions. Formation of CO_2 (mineralization) increased to about 5 %TAR during the first two weeks of aerobic incubation and then quickly slowed down under anaerobic conditions, reaching finally 6.5 % TAR after 120 days. No other volatiles were detected. The material balance was always in the range between 100.1 and 102.7% TAR.

Bentazone declined quickly from 99 % TAR at day 0 to 57 % TAR during the aerobic incubation. After that it showed only negligible further decrease under anaerobic conditions. No metabolite exceeded 1 %TAR at any sampling time. By comparison of the retention time with certified reference item, the peak eluting at 39 min could be assigned to the known soil metabolite N-methyl-bentazone (BH 351-N-Me). However, its amounts never exceeded 0.5 %TAR.

The DT_{50} for the aerobic incubation was calculated to be 18.5 days (best fit), whereas for the anaerobic phase no degradation was observed (DT_{50} > 1000 days).

I. MATERIAL AND METHODS

A. MATERIALS

1. Test item

BAS code:	BAS 351 H
Reg.No.:	51929
Chemical name (IUPAC):	3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide
Molecular weight:	240.28 g/mol (unlabelled)

^{14}C-labeled

Batch-No.:	210-2301
Position of radiolabel:	phenyl-U-^{14}C
Specific radioactivity:	5.15 MBq/mg
Radiochemical purity:	96.5% (according to certificate) 97.7% (determined by radio-HPLC prior to soil treatment

Non-labeled

Batch-No.:	01893-210
Chemical purity:	99.8%

2. Soil

A sandy loam (USDA classification) was used for the aerobic soil metabolism. After sampling, the soil was air-dried at room temperature for 5 days. Then it was passed through a 2 mm sieve. After moistening to about 10% soil moisture, it was stored at about 4°C (no longer than 3 months). The soil characteristics are summarized in Table 7.1/6.

Table 7.1/6 Properties of soil Bruch West used to investigate the degradation rate of ^{14}C-Bentazone under anaerobic conditions

Soil designation	**Bruch West (09/060/02)**
Origin	Limburgerhof, RP, Germany
DIN Particle size distribution [%] sand 0.063 – 2 mm silt 0.002 – 0.063 mm clay < 0.002 mm textural class	 58.4 29.7 11.9 loamy sand
USDA Particle size distribution [%] sand 0.050 – 2 mm silt 0.002 – 0.050 mm clay < 0.002 mm textural class	 61.0 27.1 11.9 sandy loam
Organic C [%] Organic matter [%] *	1.60 2.76
pH [H_2O] pH [$CaCl_2$]	7.9 7.1
Cation exchange capacity [$cmol^+$ / kg]	12.5
Maximum water holding capacity [g/100g dry soil]	29.3
Microbial biomass [mg C/100g dry soil]	38.7**

*organic matter = organic carbon x 1.724
**optimal glucose: 0.4%

B. STUDY DESIGN

1. Experimental conditions

The soil was adjusted to 40% MWC and left to acclimatize to 20 °C in the dark for 12 days. ^{14}C-bentazone was applied at a nominal concentration of 2.4 mg / kg dry soil, which corresponds to a field application rate of 900g/ha (assuming an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g/cm³).

For treatment, 2.45 mL of the treatment solution was applied to 2700 g soil (dry weight equivalent). The soil was mixed with a hand-mixer and aliquots were combusted to check homogeneous distribution. Portions of 111.7 g moist soil (corresponding to 100 g dry soil) were weighed into 27 glass vessels. All test vessels to be used for subsequent samplings following the 0 DAT sampling were sealed with special caps equipped with gas inlet and outlet tubes.

Several vessels were connected in series via inlet and outlet tubes and placed into a temperature-controlled chamber. The samples were incubated in the dark at a temperature of 20 °C and continuously aerated with a slight stream of moistened air. The air of each test series was led through a trapping system for volatiles consisting of a sequence of two gas washing flasks filled with ethylene glycol and 0.5 M NaOH respectively.

After 14 days, the DT_{50} of bentazone in soil was almost reached. Therefore, the soil samples were flooded with 90 mL of sterilized tap water at day 15, and the vessels incubated further under a slight stream of nitrogen in order to establish anaerobic conditions.

To monitor the conversion from aerobic to anaerobic conditions, the redox potential of the soil and water phase, as well as the pH and the O_2 content of the water phase, were measured from day 16 onwards. The redox potential became negative after additional 5 - 6 days indicating anaerobic conditions, and declined further to < - 300 mV at the end of incubation

2. Sampling

Sampling times were 0, 3, 7, 14, 21, 28, 42, 77 and 120 days after treatment (DAT).

At sampling time 0, 42 and 120 days three replicate vessels were sampled (two for workup, one for storage in freezer as back-up), whereas at all other sampling times, two vessels were taken from the incubator (one for workup, one for storage in freezer as back-up).

The trapping solutions for volatiles were sampled and checked for radioactivity at each sampling time (except day 0) and exchanged by fresh solutions.

3. Description of analytical procedures

Workup of aerobically incubated samples
The soil was transferred from the respective incubation vessel into a centrifuge tube. The soil was consecutively extracted 2 x with 100 mL methanol and 2 x with 100 mL methanol + water (1+1, v+v) on a laboratory shaker (15 min per extraction step). After centrifugation, the supernatants were filtered (paper filter) and decanted into 100 mL volumetric flasks. The flasks were made up to volume and 2 x 1 mL of each extract was measured by liquid scintillation counting (LSC).

The four corresponding extracts of one sample were pooled. The extracted soil samples were dried under a stream of nitrogen.

Workup of anaerobically incubated samples
The water-logged soil samples were transferred into centrifuge tubes using 90 mL of methanol for rinsing the test vessel. The 90 mL of methanol together with the ~ 90 mL water in the test vessel was used as first extraction solvent. After centrifugation, filtration (paper filter) and decanting of the supernatant into a 200 mL volumetric flask, the soil was further extracted 1 x with 100 mL methanol/water (1+1, v+v) and 2 x with 100 mL methanol. Each extract was filtered and decanted into a 100 mL volumetric flask. The flasks were made up to volume and each extract was checked for radioactivity by LSC measurement (2 x 1 mL aliquots).

Again, the four corresponding extracts of one sample were pooled and the extracted soil samples were dried under a stream of nitrogen.

From each pooled extract (irrespective of aerobic and anaerobic incubation), a 50 mL aliquot was concentrated to dryness by rotary evaporation (temp. < 40 °C). The residue was re-dissolved in 1.5 mL methanol/water (1+1, v+v), centrifuged and the supernatant transferred to a HPLC vial for chromatographic analysis.

For determination of the non-extractable residues, the extracted and dry soil samples were homogenized in a laboratory mill. From each homogenized sample, 4 aliquots were combusted in a Biological Oxidizer. The evolved $^{14}CO_2$ from each aliquot was trapped in Oxysolve C-400 scintillator and measured by LSC.

The volatile trapping solutions were collected at each sampling time (except day 0) and transferred into volumetric flasks. They were made up to volume and aliquots measured for radioactivity by LSC.

The procedural recoveries during the work-up (i.e. after concentration of the extracts for HPLC analysis) were checked routinely by LSC. Recoveries were always ≥ 99 %.

Calculation of the degradation rate

The degradation rates of bentazone were estimated according to the recommendations of the FOCUS kinetics workgroup.

The kinetic models proposed by the FOCUS Kinetics guidance document were considered in order to identify the most appropriate model. Conditions in the experiment changed from aerobic to anaerobic incubation between 14 and 21 days after treatment (DAT). This was accompanied by a clearly observable break in the (measured) bentazone concentration vs. time curve. Therefore, the two incubation phases (aerobic and anaerobic) of the experiment were analysed separately, using KinGUI 1.1.

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The material balance ranged from 100.1% to 102.7% TAR for all samples with an arithmetical mean of 101.3% TAR.

B. EXTRACTABLE AND BOUND RESIDUES

The distribution of the radioactive residues in the soil treated with ^{14}C-labeled bentazone at various time intervals from 0 to 120 days is shown in Table 7.1/7.

During the aerobic incubation phase, the amount of extractable radioactivity decreased rapidly from 100 %TAR at day 0 to 57 %TAR at day 14. After establishing anaerobic conditions from day 15 onwards, no significant further decline of extractability was observed. The extractable residues remained in the range of 53 - 57 %TAR until 120 days.

Corresponding to the decrease in extractability, the non-extractable ^{14}C-residues increased to 39 %TAR within the first 14 days under aerobic incubation and then remained at a rather constant level of 40 - 43 %TAR under anaerobic conditions.

C. VOLATILIZATION

Formation of CO_2 (mineralization) reached about 5 %TAR during the first two weeks of aerobic incubation and then quickly slowed down under anaerobic conditions, finally reaching only 6.5 % TAR after 120 days. No other volatiles were detected.

Table 7.1/7 Distribution of radioactivity in soil after treatment with ^{14}C-bentazone following 14 days of aerobic and 106 days of anaerobic incubation [% TAR]

Days after treatment	Extractable					NER	Volatiles		Material balance
	Methanol 1	Methanol 2	Methanol/ Water 1	Methanol/ Water 2	Total extractable		Ethylene glycol	CO_2 (NaOH)	
Aerobic pre-incubation									
0	64.7	21.5	11.2	3.3	100.7	1.9	-	-	102.7
0	63.3	22.0	11.0	3.2	99.5	2.0	-	-	101.5
0 mean	64.0	21.8	11.1	3.3	100.1	2.0	-	-	102.1
3	57.6	16.1	9.4	2.6	85.7	14.1	0.0	0.8	100.6
7	47.4	15.2	9.1	2.7	74.5	23.5	0.0	2.1	100.1
14	37.1	11.1	7.0	2.2	57.4	39.3	0.0	4.7	101.4
Days after treatment	**Extractable**					**NER**	**Volatiles**		**Material balance**
	Methanol/ Water 1	**Methanol/ Water 2**	**Methanol 1**	**Methanol 2**	**Total extractable**		**Ethylene glycol**	**CO_2 (NaOH)**	
Anaerobic incubation									
21	43.9	6.5	1.9	0.6	52.9	43.2	0.0	5.3	101.4
28	46.5	7.1	2.3	0.7	56.6	39.5	0.0	5.4	101.5
42	46.4	6.8	1.9	0.5	55.7	39.6	0.0	5.6	100.9
42	44.3	6.0	1.8	0.4	52.5	43.2	0.0	5.6	101.3
42 mean	45.4	6.4	1.9	0.5	54.1	41.4	0.0	5.6	101.1
77	47.4	6.6	1.9	0.6	56.5	40.0	0.0	6.1	102.6
120	44.6	6.7	2.4	0.7	54.4	40.0	0.0	6.5	101.0
120	43.6	7.4	2.1	0.6	53.7	41.0	0.0	6.5	101.2
120 mean	44.1	7.0	2.3	0.7	54.1	40.5	0.0	6.5	101.1

NER = non-extractable residues

TAR = total applied radioactivity (100% = 2.367 mg/kg)

D. TRANSFORMATION OF PARENT COMPOUND

The results of HPLC analysis are shown in Table 7.1/8.

The majority of extractable radioactivity always consisted of bentazone. It declined from 99% TAR at day 0 to 57% TAR at day 14 and then showed only negligible further decrease under anaerobic conditions, reaching about 53 %TAR after 120 days.

None of the other detected peaks exceeded 1 %TAR at any sampling time. By comparison of the retention time with certified reference item, the peak eluting after 39 min could be assigned to the known soil metabolite N-methyl-bentazone (BH 351-N-Me). However, its amounts never exceeded 0.4 %TAR.

Table 7.1/8 Radio-HPLC analysis of soil extracts after treatment of Bruch West soil with ^{14}C-bentazone followed by 14 days aerobic and 106 days anaerobic incubation [%TAR]

Days after treatment	^{14}C total	Unknown t_R~15-16 min	Unknown t_R~17 min	Unknown t_R~20 min	Unknown t_R~25 min	Unknown t_R~30 min	Bentazone ~33 min	BH351-N-Me ~39 min
Aerobic pre-incubation								
0	100.7			0.9		0.8	99.1	
0	99.5			0.9		0.8	97.8	
0 mean	100.1			0.9		0.8	98.5	
3	85.7			0.4		0.7	84.5	0.2
7	74.5					0.6	73.7	0.1
14	57.4					0.5	56.6	0.2
Anaerobic incubation								
21	52.9					0.6	52.0	0.3
28	56.6		0.1			0.7	55.5	0.3
42	55.7					0.7	54.7	0.3
42	52.5	0.2	0.2			0.6	51.1	0.4
42 mean	54.1	0.1	0.1			0.6	52.9	0.3
77	56.5					0.6	55.6	0.2
120	54.4	0.3				0.6	53.3	0.2
120	53.7	0.2			0.2	0.6	52.3	0.4
120 mean	54.1	0.3			0.1	0.6	52.8	0.3

~t_R = approximate retention time [min]
BH 351-N-Me (metabolite N-methyl-bentazone)
TAR = total applied radioactivity (100% = 2.367 mg/kg dry weight)
no value means "not detected"

Calculation of the degradation rate

The bentazone degradation rates for the aerobic and the anaerobic incubation phase were calculated separately.

For the aerobic phase, the FOMC kinetic model provided a better description of the experimental data than the SFO kinetic, which already showed acceptable statistical and visual fit. The DFOP kinetic model was not tested as the number of parameters of this model was close to the number of data points. Thus, persistence endpoints could be derived from the FOMC fit. Nevertheless, the SFO model provided a statistically and visually acceptable fit so that modelling endpoints could be derived from the SFO fit.

For the anaerobic phase, the SFO model provided the best description of the experimental data. Therefore, persistence and modelling endpoints were derived from the SFO fit.
The calculated DT_{50}/DT_{90} values are listed in Table 7.1/9:

Table 7.1/9 DT_{50}/DT_{90} values for bentazone incubated first under aerobic and then anaerobic conditions

Incubation phase	Kinetic model	DT_{50} [d]	DT_{90} [d]	Chi^2
Aerobic	SFO	16.0	53.1	2.2
Aerobic	FOMC	18.5	283	1.4
Anaerobic	SFO	>1000	>1000	2.3

III. CONCLUSION

Bentazone degraded rapidly under aerobic conditions by formation of non-extractable residues in soil. However, as soon as anaerobic conditions were established, the transformation slowed down considerably. No metabolites in significant amounts were detected (all peaks < 1 %TAR). The DT_{50} for the aerobic incubation was calculated to be 18.5 days (best fit, FOMC), whereas for the anaerobic phase no degradation was observed (DT_{50} > 1000 days).

7.1.3 Soil photolysis

The old soil photolysis study available was considered not to follow current guidelines anymore. Therefore, a new soil photolysis with ^{14}C-bentazone was initiated.

Report:	II A 7.1.3/1 Hassink J. 2012(a) Soil photolysis of 14C-Bentazone BASF DocID 2011/1276919
Guidelines:	EPA 161-3; EPA 835.2410; SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995); Draft OECD Guideline: Phototransformation of Chemicals in Soil Surfaces (Jan. 2002); EEC 95/36 of 14 July 1995 amending 91/414/EEC
Testing Laboratory and dates:	BASF SE; Limburgerhof; Germany Fed.Rep. 15-Mar-2011 - 09-Dec-2011
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Report:	II A 7.1.3/2 Hassink J. 2012(b) Report Amendment No. 1 - Soil photolysis of 14C-Bentazone BASF DocID 2012/1023466
Guidelines:	EPA 161-3; EPA 835.2410; SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995); Draft OECD Guideline: Phototransformation of Chemicals in Soil Surfaces (Jan. 2002); EEC 95/36 of 14 July 1995 amending 91/414/EEC
Testing Laboratory and dates:	BASF SE; Limburgerhof; Germany Fed.Rep. 27-Jan-2012 - 27-Jan-2012
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

A soil photolysis study was conducted with ^{14}C-labelled bentazone to investigate its behaviour in soil under the influence of light.

The surface of soil samples in 1 cm deep stainless steel dishes were homogeneously treated with ^{14}C-labelled bentazone and were incubated under continuous irradiation at 22°C for 15 days. Duplicate samples were taken 0, 1, 3, 7, 10 and 15 days after treatment. Dark control samples were analysed at the same sampling days. Volatiles were trapped in appropriate trapping solutions.

Soil samples were extracted with methanol and methanol/water and the extracts analysed by liquid scintillation counting (LSC) and HPLC. Bound residues were quantified by combustion and subsequent LSC measuring. The bound residues were further characterised by NaOH-extraction and distribution of radioactivity between fulvic acids, humic acids and humines.

The overall results for the material balances in the photolysis and the dark control samples were in the range of 95.3-100.3 % TAR. Carbon dioxide was the only volatile degradation product trapped with 8.1 % TAR detected after 15 days in the photolysis test and 1.8 % TAR in the dark control.

The sample extractability for the photolysis test differed from the dark control. At the end of the study about 32 % TAR were not extractable from the illuminated soil samples. About 20 % TAR were non-extractable at the end of the incubation of the dark control samples. The alkali-soluble radioactivity amounted to about 12-24 % in the period of 3 to 15 days after treatment in the soil photolysis and was further fractionated to distinguish between acid-insoluble humic acids and acid-soluble fulvic acids. The major part of the radioactivity could be assigned to the fulvic acid fraction (max. 18.7 % TAR at 7 DAT). In the dark control samples the amount of non-extractable residues was less than under light. It was confirmed that the alkali-soluble fraction (max. 12.2 % TAR at 15 DAT) consisted of radioactive material mainly assigned to the fulvic acid fraction (max. 6.3 % TAR at 15 DAT). HPLC analysis of the fulvic acid fractions showed that the radioactivity is divided between several unspecific polar peaks in negligible amounts.

The concentration of bentazone decreased to 48.7 % TAR in the course of the photolysis study and to 77.0 % in the dark control samples. No degradation products with more or equal 1 % resp. 4% TAR occurred in the photolysis samples or in the dark controls.

The half-live values (SFO DT_{50}) for bentazone in the test systems were calculated to be 12.8 days under continuous irradiation and 42.1 days in the dark

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

BAS code:	BAS 351 H
Reg.No.:	51929
CAS-No.:	25057-89-0
Chemical name (IUPAC):	3-isopropyl-1H-2,1,3,-benzothiadiazin-4(3H)-one-2,2-dioxide
Molecular weight:	240.28 g/mol (unlabelled)
Position of radiolabel:	phenyl-U-^{14}C
Batch-No.:	210-2301
Specific radioactivity:	5.15 MBq/mg (309000 dpm/µg)
Radiochemical purity:	96.5%

2. Soil

A sandy loam (according to USDA) was used for the soil photolysis. The soil was freshly sampled from the field, passed through a 2 mm sieve, and stored for no longer than 3 months at 4°C before use. The soil was pre-incubated at 22°C for 24 hours before application. A summary of the soil characteristics is given in Table 7.1/10.

Table 7.1/10 Properties of soil Bruch West used to investigate degradation of [phenyl-U-^{14}C]- bentazone under photolytic conditions

Soil designation	**Bruch West (11/060/01)**
Origin	Limburgerhof, Germany
DIN Particle size distribution [%] sand 0.063 – 2 mm silt 0.002 – 0.063 mm clay < 0.002 mm textural class	 66.2 24.5 9.2 loamy sand
USDA Particle size distribution [%] sand 0.050 – 2 mm silt 0.002 – 0.050 mm clay < 0.002 mm textural class	 69.1 21.6 9.2 sandy loam
Organic C [%] Organic matter [%] *	1.85 3.19
pH (H_2O) pH ($CaCl_2$)	7.8 7.2
Cation exchange capacity [$cmol^+$ / kg]	12.3
Maximum water holding capacity [g/100g dry soil]	33.4
Microbial biomass [mg C/100g dry soil]	63.1

* organic matter = organic carbon x 1.724*

B. STUDY DESIGN

1. Experimental conditions

For each test (photolysis and dark control), 10 small steel dishes (88 mm x 43 mm x 12 mm) were filled with soil almost up to the rim and arranged in a rectangular bowl with a connected thermostat. The soil surfaces were then treated with the radio-labelled test item. The temperature was adjusted and controlled with the help of a respective probe placed in untreated soil in an additional 11th dish (only photolysis). The bowl was closed airtight with a quartz glass covering. The head space of the bowl was continuously purged with moistened and CO_2-free air during the incubation under irradiated conditions. The exiting air was flushed through three gas washing flasks containing ethylene glycole, H_2SO_4 (0.5M) and NaOH (0.5M) in order to collect potentially evolving ^{14}C-volatiles.

For treatment, the soil was adjusted to 50% of the maximum water holding capacity before being filled into the dishes. 216 µg dissolved in 330 µL of an acetonitrile stock solution was pipetted as far as possible homogeneously distributed onto the soil surface. This resulted in an application rate of 7.207 µg/g dry soil, corresponding to about 1081 g/ha field application rate when assuming an equal distribution in a soil depth of 1 cm and a soil density of 1.5 g/cm^3.

The incubation bowl was placed under a SUNTEST CPS plus (*Atlas*) equipped with a Xenon lamp emitting light with a sunlight similar spectrum at a light intensity of about 3 mW/cm^2. This corresponds to a clear summer day in Southern Germany (about 48°N). Wavelengths < 290 nm were filtered off.

The incubation duration was 15 days under continuous irradiation. The soil temperature during incubation was kept at 22 ± 1°C.

The equipment used for incubation of the dark control samples was the same as used for the photolysis experiment, except that the incubation device was placed in a climatic chamber at 22 ± 1°C without thermostat connection.

2. Sampling

Sampling times were at 0 (no sampling of volatiles), 1, 3, 7, 10 and 15 days after treatment for the photolysis experiment as well as for the dark control incubations. At each sampling time, the respective trapping solutions were removed and replaced by fresh ones. The 0 day samples were the same for the photolysis and the dark controls.

3. Analytical procedures

Each soil sample was extracted immediately after sampling: three times with 60 ml methanol, and three times with 60 ml of methanol/water (1:1), respectively. For each extraction step, the suspension was shaken at 180 rpm for 15 min. Solid and liquid phase were separated by centrifugation and the supernatant filtered. The three corresponding methanol and three methanol/water extracts were pooled, adjusted to a defined volume of 200 ml each and measured for radioactivity (LSC). The combined extracts were used directly for HPLC-analysis without further work-up.

The extracted soil samples were air dried. To determine the amount of non-extractable residues (NER) the soil was homogenized in a laboratory mill and aliquots thereof were combusted. The evolved $^{14}CO_2$ from each combusted aliquot was trapped in Oxysolve C400 scintillator and measured by LSC.

Since the non-extractable residues (NER) exceeded 10 % TAR in samples of the photolysis and the dark control test, the distribution of radioactivity in humic acids, fulvic acids and humin was further investigated. The soil samples were extracted three times with 50 ml 0.5 M NaOH on a rotary shaker (overnight, overday, overnight) and then centrifuged. The supernatant containing the NaOH soluble fulvic and humic acids was filtered and decanted into a volumetric flask. The soil residue was washed two times with 25 ml bidest. water and the water was filtered and decanted into a volumetric flask. All extracts were analysed by LSC and finally pooled. In order to quantify the amount of radioactivity left in the insoluble humines, the soil residue after NaOH extraction was air-dried, homogenized and the weight determined. Three aliquots were combusted and analysed by LSC.

The humic acids were precipitated from the NaOH extracts by adjusting the pH to 1 - 1.5 with conc. HCl (37 %), leaving the fulvic acids in solution. To finish the precipitation completely, the phases were stored over the weekend in a refrigerator and finally centrifuged and decanted into a graduated cylinder. The precipitated residues were dissolved in 0.5 M NaOH. All liquid phases were measured by LSC. The fulvic acid fractions were analysed by HPLC.

4. Kinetic modelling

The measured residues of the applied substance were analysed in order to determine the degradation kinetics and trigger endpoints for persistence (DT_{50}, DT_{90}) following the recommendations of the FOCUS Kinetics workgroup [*FOCUS (2006)*].

II. RESULTS AND DISCUSSION

A. MASS BALANCE

The overall mean values for the material balance in the photolysis and the dark control were in the range of 95.3-101.0 % TAR (Table 7.1/11, Table 7.1/12)

B. EXTRACTABLE AND BOUND RESIDUES

The sample extractability for the photolysis test differed from the dark control (Table 7.1/11 and Table 7.1/12). At the end of the study about 32 % TAR were not extractable from the illuminated soil samples. About 20 % TAR were non-extractable at the end of the incubation of the dark control samples.

Table 7.1/11 Distribution of radioactivity in soil Bruch West after treatment with [phenyl-U-^{14}C]-bentazone and incubation under irradiated conditions [%TAR]

DAT	MeOH	MeOH/H_2O	ERR	NER	Volatiles*	Sum
0 DAT I	95.9	4.5	100.4	1.0	n.a.	101.4
0 DAT II	93.6	4.4	98.0	0.6	n.a.	98.6
0 DAT mean	94.7	4.4	99.2	0.8	n.a.	100.0
1 DAT I	84.0	7.9	91.9	7.3	0.5	99.7
1 DAT II	86.7	7.3	94.0	7.8	0.5	102.3
1 DAT mean	85.3	7.6	92.9	7.5	0.5	101.0
3 DAT I	68.3	10.3	78.6	17.3	2.2	98.1
3 DAT II	66.9	11.8	78.7	17.7	2.2	98.6
3 DAT mean	67.6	11.0	78.7	17.5	2.2	98.4
7 DAT I	42.3	15.9	58.2	32.9	5.0	96.1
7 DAT II	39.4	16.2	55.7	33.9	5.0	94.6
7 DAT mean	40.9	16.1	56.9	33.4	5.0	95.4
10 DAT I	45.8	17.9	63.6	28.8	6.5	98.9
10 DAT II	45.2	14.4	59.6	28.5	6.5	94.6
10 DAT mean	45.5	16.1	61.6	28.6	6.5	96.8
15 DAT I	47.2	13.9	61.1	28.7	8.1	97.9
15 DAT II	35.1	14.2	49.3	35.2	8.1	92.6
15 DAT mean	41.2	14.1	55.2	31.9	8.1	95.3

ERR = extractable radioactive residues
NER = non-extractable radioactive residues
* only CO_2 was found

Table 7.1/12 Distribution of radioactivity in soil Bruch West after treatment with [phenyl-U-^{14}C]-bentazone and incubation under dark conditions [%TAR]

DAT	MeOH	MeOH/H_2O	ERR	NER	Volatiles*	Sum
0 DAT I	95.9	4.5	100.4	1.0	n.a.	101.4
0 DAT II	93.6	4.4	98.0	0.6	n.a.	98.6
0 DAT mean	94.7	4.4	99.2	0.8	n.a.	100.0
1 DAT I	92.9	3.8	96.7	4.2	0.3	101.2
1 DAT II	91.4	3.7	95.1	4.0	0.3	99.3
1 DAT mean	92.2	3.8	95.9	4.1	0.3	100.3
3 DAT I	91.7	3.0	94.7	6.0	0.6	101.2
3 DAT II	89.9	2.7	92.6	5.7	0.6	99.0
3 DAT mean	90.8	2.8	93.7	5.8	0,6	100.1
7 DAT I	82.1	3.1	85.2	11.7	1.1	97.9
7 DAT II	84.2	3.2	87.4	10.3	1.1	98.8
7 DAT mean	83.2	3.2	86.3	11.0	1.1	98.3
10 DAT I	79.8	3.8	83.6	14.0	1.4	99.0
10 DAT II	81.5	4.1	85.7	13.7	1.4	100.7
10 DAT mean	80.7	4.0	84.7	13.9	1.4	99.9
15 DAT I	71.7	4.3	75.9	20.8	1.8	98.6
15 DAT II	73.0	5.0	78.0	18.4	1.8	98.3
15 DAT mean	72.3	4.6	77.0	19.6	1.8	98.4

ERR = extractable radioactive residues
NER = non-extractable radioactive residues
* only CO_2 was found

C. VOLATILIZATION

Carbon dioxide was the only volatile degradation product trapped. In the sodium hydroxide traps 8.1% TAR were detected after 15 days in the photolysis test and 1.8% TAR after 15 days in the dark control. In H_2SO_4 and ethylene glycol no significant radioactivity could be measured at any time point.

D. TRANSFORMATION OF PARENT COMPOUND

The results of the HPLC analysis are shown in Table 7.1/13 and Table 7.1/14.

The concentration of the test item bentazone decreased to 48.7 % TAR in the course of the photolysis study and to 77.0 % in the dark control samples. Hence, dissipation of the test item in the photolysis samples was more rapid than in the dark. No degradation products with more or equal than 4 % resp. 1 % TAR occurred in the photolysis samples and the dark controls.

Table 7.1/13 Radio-HPLC-analysis of soil extracts after treatment of soil Bruch West with [phenyl-U-^{14}C]-bentazone and incubation under irradiated conditions [%TAR]

days after treatment	Bentazone	others*	sum
0 DAT I	99.6	0.8	100.4
0 DAT II	96.8	1.2	98.0
0 DAT mean	98.2	1.0	99.2
1 DAT I	91.2	0.7	91.9
1 DAT II	92.6	1.4	94.0
1 DAT mean	91.9	1.0	92.9
3 DAT I	76.7	1.9	78.6
3 DAT II	76.9	1.8	78.7
3 DAT mean	76.8	1.8	78.7
7 DAT I	52.7	5.5	58.2
7 DAT II	51.9	3.8	55.7
7 DAT mean	52.3	4.6	56.9
10 DAT I	59.9	3.7	63.6
10 DAT II	59.6	0.0	59.6
10 DAT mean	59.7	1.9	61.6
15 DAT I	57.3	3.9	61.1
15 DAT II	40.2	9.1	49.3
15 DAT mean	48.7	6.5	55.2

* each single peak below 4% TAR

Table 7.1/14 Radio-HPLC-analysis of soil extracts after treatment of soil Bruch West with [phenyl-U-^{14}C]-bentazone and incubated under dark conditions [%TAR]

days after treatment	**Bentazone**	**others***	**sum**
0 DAT I	99.6	0.8	100.4
0 DAT II	96.8	1.2	98.0
0 DAT mean	98.2	1.0	99.2
1 DAT I	96.7	0.0	96.7
1 DAT II	95.1	0.0	95.1
1 DAT mean	95.9	0.0	95.9
3 DAT I	94.1	0.6	94.7
3 DAT II	92.2	0.4	92.6
3 DAT mean	93.2	0.5	93.7
7 DAT I	85.2	0.0	85.2
7 DAT II	87.4	0.0	87.4
7 DAT mean	86.3	0.0	86.3
10 DAT I	83.6	0.0	83.6
10 DAT II	85.7	0.0	85.7
10 DAT mean	84.7	0.0	84.7
15 DAT I	75.9	0.0	75.9
15 DAT II	78.0	0.0	78.0
15 DAT mean	77.0	0.0	77.0

* each single peak below 1% TAR

Characterization of non-extractable residues

Since the bound residues in soil amounted up to 35% TAR, the already solvent extracted soil was further extracted with NaOH.

The alkali-soluble radioactivity amounted to about 12-24 % in the period of 3 to 15 days after treatment in the soil photolysis. This alkali-soluble radioactivity was further fractionated to distinguish between acid-insoluble humic acids and acid-soluble fulvic acids. The major part of the radioactivity could be assigned to the fulvic acid fraction (max. 18.7 % TAR at day 7) which consisted of several unspecific peaks in negligible amounts (Table 7.1/15, Table 7.1/16).

In the dark control samples the amount of non-extractable residues was less than under light (Table 7.1/17, Table 7.1/18). It was confirmed that the alkali-soluble fraction (max. 12.2 % TAR at day 15) consisted of radioactive material mainly assigned to the fulvic acid fraction (max. 6.3 % TAR at day 15).

Table 7.1/15 Characterization of non-extractable residues in soil Bruch West after treatment with ^{14}C-bentazone and incubation under irradiated conditions [%TAR]

DAT	% TAR					
	NER initial	NaOH extraction	Water extraction	Sum of NaOH and water extracts	Soil residues after extraction (humin)	Sum*
3 I	17.33	11.28	0.24	11.52	2.81	14.33
3 II	17.66	12.04	0.36	12.40	3.04	15.44
3 mean	17.50	11.66	0.31	11.96	2.92	14.88
7 I	32.94	21.92	0.54	22.46	5.67	28.13
7 II	33.88	22.88	0.74	23.63	5.04	28.67
7 mean	33.41	22.39	0.65	23.04	5.35	28.40
10 I	28.76	18.45	0.51	18.95	6.47	25.42
10 II	28.48	19.29	0.49	19.78	5.22	25.00
10 mean	28.62	18.87	0.50	19.37	5.84	25.21
15 I	28.69	21.79	1.27	23.07	5.54	28.61
15 II	35.21	23.66	1.35	25.01	7.80	32.80
15 mean	31.95	22.73	1.31	24.04	6.67	30.71

*deviations from initial NER values have to be attributed to differing LSC results

Table 7.1/16 Distribution of radioactivity between fulvic acids and humic acids in soil Bruch West after treatment with ^{14}C-bentazone and incubation under irradiated conditions [%TAR]

DAT	% TAR			
	Sum of NaOH and water extracts	Fulvic acid	Humic acid	Sum*
3 I	11.52	8.52	3.17	11.69
3 II	12.40	9.20	3.37	12.57
3 mean	11.96	8.86	3.27	12.13
7 I	22.46	18.39	4.72	23.11
7 II	23.63	19.09	3.90	22.99
7 mean	23.04	18.74	4.31	23.05
10 I	18.95	15.48	3.20	18.68
10 II	19.78	16.64	2.97	19.61
10 mean	19.37	16.06	3.09	19.15
15 I	23.07	15.30	5.94	21.24
15 II	25.01	16.71	6.26	22.96
15 mean	24.04	16.01	6.10	22.10

* slight deviations from initial values have to be attributed to differing LSC results

Table 7.1/17 Characterization of non-extractable residues in soil Bruch West after treatment with ^{14}C-bentazone and incubation under dark conditions [%TAR]

DAT	% TAR					
	NER initial	NaOH extraction	Water extraction	Sum of NaOH and water extracts	Soil residues after extraction (humin)	Sum*
7 I	11.67	5.39	0.20	5.60	2.99	8.59
7 II	10.29	5.10	0.19	5.29	3.28	8.57
7 mean	10.98	5.25	0.19	5.44	3.14	8.58
10 I	13.97	6.16	0.21	6.37	4.24	10.60
10 II	13.74	5.86	0.18	6.03	4.47	10.50
10 mean	13.86	6.01	0.20	6.20	4.35	10.55
15 I	20.79	11.47	1.00	12.48	6.62	19.09
15 II	18.45	10.98	1.01	11.98	6.18	18.16
15 mean	19.62	11.23	1.00	12.23	6.40	18.63

*deviations from initial NER values have to be attributed to differing LSC results

Table 7.1/18 Distribution of radioactivity between fulvic acids and humic acids in soil Bruch West after treatment with ^{14}C-bentazone and incubation under dark conditions [%TAR]

DAT	% TAR			
	Sum of NaOH and water extracts	Fulvic acid	Humic acid	Sum*
7 I	5.60	4.24	1.41	5.65
7 II	5.29	4.00	1.45	5.45
7 mean	5.44	4.12	1.43	5.55
10 I	6.37	5.04	1.42	6.46
10 II	6.03	4.70	1.51	6.21
10 mean	6.20	4.87	1.46	6.33
15 I	12.48	6.45	4.07	10.52
15 II	11.98	6.14	4.47	10.61
15 mean	12.23	6.30	4.27	10.56

* slight deviations from initial values have to be attributed to differing LSC results

The distribution of radioactivity over all fractions of humic substances confirm the overall knowledge about bentazone that it is tightly incorporated into the organic soil matrix and cannot be released as bentazone even with harsh extraction solvents like 0.5 N NaOH.

E. KINETIC MODELING RESULTS

The half-live values (SFO DT_{50}) for bentazone in the test systems were calculated to be 12.8 days under continuous irradiation and 42.1 days in the dark.

Although the FOMC model showed a better fit for both data sets (irradiated soil and the dark control), the fitted kinetic parameters were not significant on a 5 % level. Therefore, the SFO values were regarded as more suitable to describe bentazone degradation in soil.

Table 7.1/19 Estimated DT_{50} and DT_{90} values of bentazone obtained in soil photolysis study

Kinetic model	Substance	DT_{50} [d]	DT_{90} [d]	χ^2 - error level [%]	Visual fit
SFO	soil photolysis	12.8	42.4	7.6	acceptable
SFO	dark control	42.1	139.7	1.0	acceptable

III. CONCLUSION

The results of the present study showed that sunlight may have an influence on the degradation rate of bentazone in soil. The incorporation into the humic substances was observed to be faster under irradiated than under dark conditions. However, no photodegradates were formed in significant amounts (all peaks < 4%).

Overall Summary on Route of Degradation in Soil

The overall understanding on the route of degradation of bentazone in soil was confirmed by the studies performed according to the newest guidelines and analytical techniques. The major route of degradation in soil is formation of bound residues with tight incorporation into all fractions of the organic soil matrix (up to 64% of the applied radioactivity distributed between fulvic acids, humic acids and humins after 120 days). First metabolisation step is assumed to be hydroxylation and the phenyl-moiety. The resulting 6- or 8-OH-bentazones are then quickly incorporated into the humic substances. Mineralization reached 8% within 120 days.

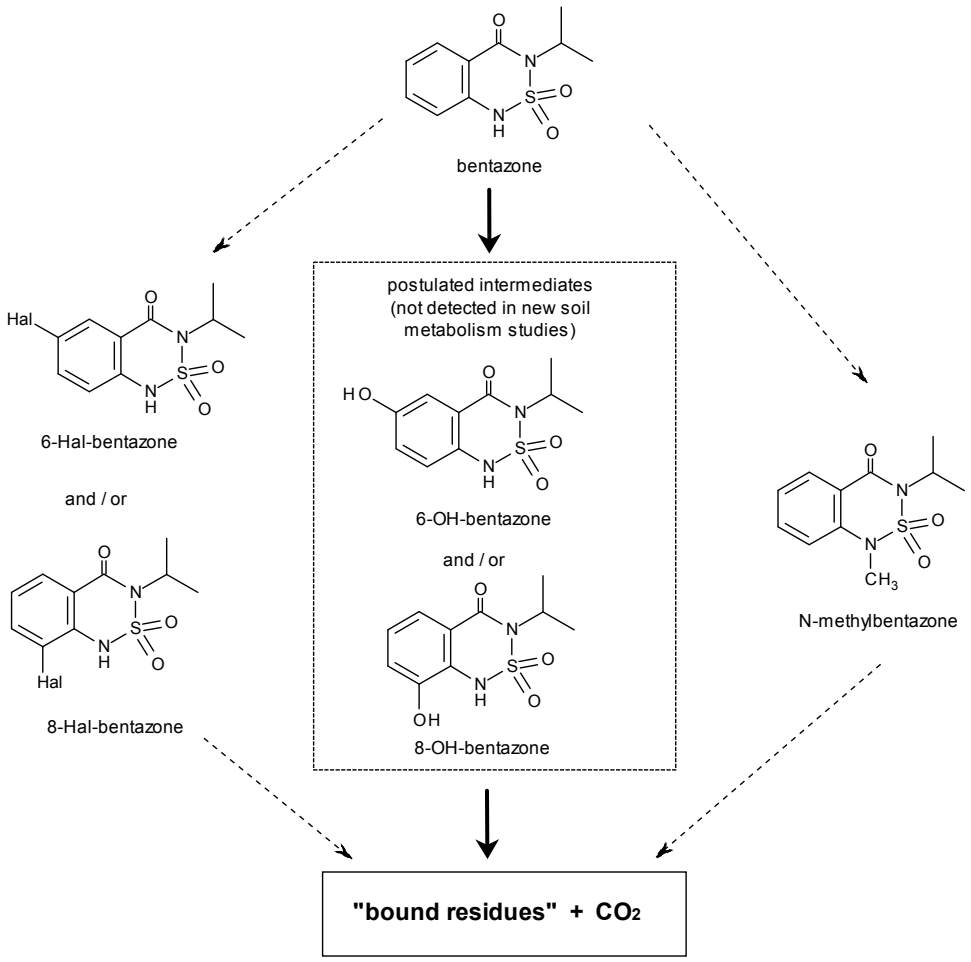
The degradation is considerably slowed down under anaerobic conditions. The new soil photolysis study showed a faster degradation under irradiated conditions compared to dark conditions, however, no new metabolites appeared. It can be concluded that the route of degradation is still the same under sunlight, only the formation rate of bound residues seems to be enhanced.

N-methyl-bentazone was the only metabolite reaching barely 5% of the initially applied radioactivity (see also chapter 7.2 Rate of Degradation) during aerobic incubation. No other peak exceeded 4% in any of the new bentazone studies (including soil photolysis). Halogenated bentazone derivatives were not identified. If formed at all, they were among those minor peaks < 4% AR.

Leaching evaluation and exposure assessments for ecotoxicological risk assessments according to current guidelines and guidance documents were therefore performed for the active ingredient bentazone and its metabolite N-methyl-bentazone.

An updated route of degradation of bentazone in soil in shown in Figure 7.1/2.

Figure 7.1/2 Proposed route of bentazone degradation in soil (update 2012)



Hal = halogenated bentazone (Cl or Br, only detected in older studies)

7.2 Rate of degradation in soil(s) - laboratory studies

A summary of already peer-reviewed DT50 values from the previous Annex I evaluation as listed in the EU review report (2000) is given below. The degradation rates of bentazone under laboratory conditions showed a rather high variability. DT_{50} values ranged from 8 to 102 d with an average of 45 days according to the EU review report. In the “Monograph of Bentazone, 17 Sept 1996”, the basis of the laboratory rate of degradation endpoints is given in the summary table shown in Table 7.2/1.

Table 7.2/1 Laboratory studies listed in the Bentazone Monograph used for the endpoints of the EU review report on bentazone (2000)

Summary: Rate of degradation in laboratory studies

Calculation of DT50 and DT90 values (days)*) of bentazone :

Soil no.	appl. rate mg/kg	pH	Moisture %	Temperature °C	$DT50_l$ d	$DT90_l$ d	Ref.
1	2	6.7	15	22 +/- 2	12	39	(1)
1	5	6.7	15	22 +/- 2	25	-	(1)
Influence of Moisture							
1	2	6.7	5	22 +/- 2	20	66	(1)
1	2	6.7	10	22 +/- 2	18	59	(1)
1	2	6.7	15	22 +/- 2	23	76	(1)
Influence of Temperature							
1	2	6.7	12	8-10	161**)	-	(1)
1	2	6.7	12	22+/- 2	35**)	-	(1)
1	2	6.7	12	35-37	35**)	-	(1)
Influence of pH							
4	2	6.4	12	22 +/- 2	34	113	(1)
4	2	4.6/5.5	12	22 +/- 2	10	33	(1)
2	2	7.5	20	22 +/- 2	56	-	(1)
2	3/3	7.5	20	22 +/- 2	70/84	-	(3)
3	2	5.1	20	22 +/- 2	102	-	(1)
4	3/3/3	4.6	12	22 +/- 2	42/46/47	-	(2)
5	10	6.1	52	22 +/- 2	65	215	(4)
6	10	5.0	31	22 +/- 2	45	151	(4)
7	10	7.7	61	22 +/- 2	45	150	(4)
9	5	6.8	40	20	38	125	(5)
10	5	5.2	40	20	16	54	(5)
11	2	7.2	40	20	11	37	(6)
12	2	6.7	40	20	47	198(extr.)	(6)
13	2	7.1	40	20	80	-	(6)
14	2	5.8	40	20	8	85	(6)

*) All calculation were conducted according (7)(8)
-if not stated otherwise.
**) Graphical interpolation

References: (1) Drescher und Otto, 1972, BOD96-00054 (2) Drescher and Otto, 1973, BOD96-00055, BOD96-00057 (3) Drescher and Otto, 1973, BOD96-00056 (4) Keller, 1987, BOD95-00264 (5) Anonym, 1974, BOD96-00058 (6) Keller, 1988, BOD96-00124 (7) Timme and Frehse, 1980, BOD96-00138 (8) Timme et al., 1986, BOD96-00139.

The basis of the studies and the selection of mean parameters are the following references:

1. Drescher and Otto (1972). Studies on the degradation of bentazone (BAS 351-H) in soil. BASF DocID 1972/0030.
2. Drescher and Otto (1973). Studies on the degradation of bentazone (BAS 351-H) in soil – 2nd report. BASF DocID 1973/0031.
3. Drescher and Otto (1973). Über den Abbau von Bentazon im Boden (4. Mitteilung). BASF DocID 1973/0047.
4. Anonym (1974). Verhalten des Pflanzenschutzmittelwirkstoffes im Boden. BASF DocID 1974/10086.
5. Keller, E. (1987). The aerobic soil metabolism of BAS 351 H (Bentazone). BASF DocID 1987/0415.
6. Keller, W. (1988). Degradation behavior of Bentazone in soil. BASF DocID 1988/10121.

However, the studies that are mentioned in the Monograph were not carried out according to current OECD guideline (OECD, 307). Old analytical methods were used and for some studies the measured concentrations were not even given in the report but only shown as graphs.

Therefore a new rate of degradation study was initiated.

One additional study is available which was already peer reviewed in the EU process and included in the "Addendum to the Monograph – Volume 3, 17 May 2000": Ebert (2000) Degradation of Bentazon (BAS 351 H) in lysimeter soil (Borstel, Northern Germany). BASF DocID 2000/1000142.

As supplemental information, two scientific publications dealing with the influence of simultaneous application of atrazine and with the influence of soil depth and with spatial variations on the degradation rate of bentazone in soil are also summarized below. Overall, they show that degradation rates for bentazone in soil found in public literature are in the same range as found in BASF laboratory soil degradation studies.

7.2.1 Aerobic degradation of the active substance in soils at 20°C

Report:	II A 7.2.1/1 Tornisielo A., Sacchi R.R. 2011(b) Rate of degradation of BAS 351 H in European soils under aerobic conditions BASF DocID 2011/1000621
Guidelines:	OECD 307 (2002)
Testing Laboratory and dates:	BASF SA; Guaratingueta; Brazil 19-Feb-2009 - 06-Jul-2009
GLP:	Yes (laboratory certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Executive Summary

The degradation rate of BAS 351 H (bentazone) under aerobic conditions was investigated in four different soils at a temperature of 20°C.

The soils were typical agricultural soils from Germany, freshly collected from the field and passed through a 2 mm sieve before use. The soils were treated with a nominal rate of 2.0 mg ^{14}C-labeled BAS 351 H per kg dry soil which corresponds to a field application rate of 750 g bentazone / ha, assuming equal distribution in the upper 2.5 cm soil layer and a soil density of 1.5 g/cm^3.

The incubations were carried out under dark conditions at soil moisture of 40 % of the maximum water holding capacity. A closed incubation system with continuous aeration (moistened air) was used with an attached trapping system for the determination of volatile compounds. Samples were taken at 0, 3, 7, 14, 29, 62, 90, and 120 days after treatment (DAT). The soil samples were extracted twice with methanol and four times with methanol/water (1:1) and the extracts analysed by means of liquid scintillation counting (LSC) and HPLC. The amount of non-extractable residues was determined by combustion and subsequent LSC measurements.

The mass balance throughout the study ranged from 91.8 to 113.2 % TAR with average values of 98.3 to 100.3 % TAR for each soil. The extractable radioactivity decreased from 93.8 - 100.9% TAR (total applied radioactivity) at day 0 to 6.9 - 19.4% after 120 days. The majority of radioactivity in the extracts was always unchanged test item. At the end of incubation, bentazone was detected in amounts of 4.8 - 18.8 % TAR.

Three metabolites appeared in the chromatograms. By comparison of retention times with the reference substance, one metabolite could be assigned to the known soil metabolite N-methyl-bentazone (M351H009). It reached maximum amounts of about 5 % TAR in soil LUFA 2.2. In all other soils, it never exceeded 2.3 % TAR. The other two unknown compounds never exceeded 2.2 % TAR at any sampling time. Formation of CO_2 was observed in all four soils reaching in total 9.1 to 21.2 % TAR after 120 days. No other volatile compounds were detected. Non-extractable residues were formed in high amounts with a maximum of 63.8 to 92.5 % TAR at the end of the study.

Dissipation times of BAS 351 H were calculated with the computer program KinGUI 1.1, applying single first order kinetics (SFO) and first order multi compartment kinetics (FOMC). The first order half-lives are shown in the table below.

Soil	DT_{50} [days]	DT_{90} [days]	Type I error rate (Prob.>t)	χ^2 error level	Visual fit
Bruch West	33.0	109.6	9.1×10^{-13}	1.77	excellent
Li 10	43.4	144.2	5.4×10^{-13}	1.38	excellent
LUFA 2.2	30.9	102.7	2.5×10^{-7}	6.65	excellent
LUFA 2.3	49.1	163.2	4.9×10^{-10}	2.52	excellent

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

BAS code: BAS 351 H
Reg.No.: 51929
Chemical name (IUPAC): 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide
Molecular weight: 240.28 g/mol (unlabeled)
Batch No.: 210-2201
Position of radiolabel: U-^{14}C
Specific radioactivity: 5.29 MBq/mg
Radiochemical purity: 97.3 % (according to certificate)
99.09 % (determined by radio-HPLC prior to soil treatment in study)

2. Soils

Four different soils were used for treatment and incubation. Prior to use, all soils were passed through a 2 mm sieve. Before application, the soils were acclimatized for 12 days in the dark at ambient temperature and soil moisture of approximately 40 % of the maximum water holding capacity. The soil characteristics are summarized in Table 7.2/2.

Table 7.2/2 Properties of soils used to investigate degradation rate of bentazone

Soil designation	Bruch West (09/060/01)	Li 10 (09/1680/01)	Lufa 2.2 (09/736/01)	Lufa 2.3 (09/570/01)
Origin	Limburgerhof, RP, Germany	Limburgerhof, RP, Germany	Hanhofen, RP, Germany	Offenbach, RP, Germany
DIN Particle size distribution [%]				
sand 0.063 – 2 mm	60.3	79.2	84.5	75.9
silt 0.002 – 0.063 mm	28.0	15.3	10.7	17.7
clay < 0.002 mm	11.7	5.6	4.8	6.4
textural class	loamy sand	loamy sand	silty sand	loamy sand
USDA Particle size distribution [%]				
sand 0.050 – 2 mm	63.7	80.2	85.6	77.1
silt 0.002 – 0.050 mm	24.6	14.3	9.6	16.5
clay < 0.002 mm	11.7	5.6	4.8	6.4
textural class	sandy loam	loamy sand	loamy sand	sandy loam
Organic C [%]	1.37	0.97	0.15	0.98
Organic matter [%] *	2.36	1.67	0.26	1.69
pH [H_2O]	7.8	6.8	6.2	7.4
pH [$CaCl_2$]	7.1	6.1	5.7	6.7
Cation exchange capacity [$cmol^+$ / kg]	10.1	4.7	5.5	7.2
Maximum water holding capacity [g/100g dry soil]	28.2	25.7	33.8	25.0
Microbial biomass - before start of study [mg C/100g dry soil]	37.0**	19.4**	34.1**	19.4**
Microbial biomass - 62 DAT [mg C/100g dry soil]***	34	24	26	19
Microbial biomass - 120 DAT [mg C/100g dry soil]***	41	27	27	34

* organic matter = organic carbon x 1.724

** optimal glucose: 0.2%

*** determined at test facility

RP Rhineland-Palatinate

B. STUDY DESIGN

1. Experimental conditions

The soils were treated at a nominal concentration of 2.00 mg ^{14}C-bentazone per kg dry soil, which corresponds to a field application rate of 750 g bentazone / ha, calculated on the basis of an equal distribution in the top 2.5 cm soil layer and a soil density of 1.5 g/cm³.

An application solution was prepared in methanol by mixing non-labelled bentazone and ^{14}C-labeled bentazone. For treatment, appropriate amounts of the application solution with a concentration of 0.398 mg bentazone / mL and a specific radioactivity of 96818.1 dpm / µg were used.

The treated soils were homogenized using a household mixer, and soil portions of 50.0 g dry soil equivalents were filled into suitable test vessels. The number of vessels was sufficient to allow duplicate sampling at each sampling time and to keep a limited number of reserve vessels.

The vessels were placed into a temperature-controlled chamber (incubation cabinet) and incubated in the dark for 120 days at a temperature of 20°C. Throughout the incubation period, the test vessels were continuously aerated with a slight stream of moistened and CO_2-free air. The outgoing air was led through a series of gas-washing bottles containing trapping solutions for potential volatiles (0.5 M NaOH, 0.5 M H_2SO_4, ethylene glycol). The water content of the soils was monitored throughout the incubation period by weighing representative vessels at each sampling time.

For determination of the microbial biomass during and at the end of incubation, additional soil samples treated with similar amounts of pure methanol were incubated under the same conditions.

2. Sampling
Sampling times were 0, 3, 7, 14, 29, 62, 90 and 120 days after treatment (DAT).

At 0 DAT as well as at 62 DAT and 120 DAT two replicate samples were worked up. At the other sampling times only one replicate was worked up, the second one was stored in a freezer.

3. Description of the analytical procedures

After sampling, the soil was removed from the respective incubation vessel and filled into a plastic centrifuge tube. It was then extracted twice with 100 mL methanol and four times with 100 mL methanol+water 1:1 (v:v) on a laboratory shaker at 250 rpm for about 30 minutes, respectively. After each extraction step the sample was centrifuged and the supernatant measured by LSC.

After the last methanol+water extraction, the soil was rinsed with 100 mL acetone. The acetone was also checked for radioactivity.

The combined methanol extracts as well as the combined methanol+water extracts and the acetone extracts were concentrated to a small volume by rotary evaporation (temp. ~40°C). The concentrated extracts were sonicated to ensure that all radioactivity was dissolved. Prior to HPLC analysis, the concentrated extracts were centrifuged. The supernatant was radioassayed and analysed by HPLC to obtain the metabolite pattern.

The extracted soil was dried at room temperature and homogenized with a laboratory mill. At least three aliquots per soil and sampling date were combusted to determine the amount of the non-extractable radioactive residues (NER).

The microbial biomass of the soils (treated with methanol only) was determined after 62 and 120 days of incubation. The method was based on the determination of the oxygen consumption upon addition of glucose according to Anderson & Domsch [*J.P.E. Anderson and K.H. Domsch, Soil Biol. Biochem. Vol.10, 215-221, 1978].*

4. Kinetic modelling

Kinetic analysis and calculations of DT_{50} and DT_{90} values for bentazone were performed following the recommendations of the FOCUS Kinetics workgroup [*FOCUS (2006) "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434pp.*]. The analysis was conducted by non-linear regression methods employing the software tool KinGUI 1.1 [*SCHÄFER, D., MIKOLASCH, M., RAINBIRD, P., HARVEY, B. (2007) KinGUI: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics. In: Del Re, A.A.M. et al. (Eds.): Proceedings of the XIII Symposium on Pesticide Chemistry, Piacenza, 2007, P. 916-923*].

For each data set, the kinetic models proposed by the FOCUS Kinetics guidance document were tested in order to identify the best-fit model. The goodness-of-fit was evaluated by visual assessment, χ^2 minimum error and type-I-error rate (t-test) (for details see Chapter 6.3 in FOCUS (2006)).

In all soils, the measured values at DAT 0 and all later sampling time points (1, 3, 7, 14, 29, 62, 90 and 120) were set to the amount of extractable residues. Replicate measurements where available were used for parameter estimation.

II. RESULTS AND DISCUSSION

The distribution of radioactive residues in the different soils treated with ^{14}C-labeled bentazone at various time intervals from 0 DAT to 120 DAT is shown in Table 7.2/3 to Table 7.2/6.

A. MASS BALANCE

The material balance ranged from 91.8% to 111.8% TAR for all soils and samples with average values of 98.6 % (Bruch West), 98.3 % (Li 10) and 100.3 % TAR (LUFA 2.2) and 98.6 % (LUFA 2.3) throughout the incubation period of 120 days.

B. EXTRACTABLE AND BOUND RESIDUES

The extractability of the radioactive residues decreased from 96.4 - 100.9 % TAR at 0 DAT to about 6.9 - 19.4 % TAR after 120 days of incubation. For all samples, the predominant part of the radioactivity could be extracted with methanol (91.3 to 3.1 % TAR). The subsequent extraction with methanol / water yielded additional 3.8 to 9.6 % TAR. The acetone fractions obtained by rinsing the soil with acetone after the last extraction step contained only negligible amounts of radioactivity (≤ 0.8 % TAR).
Non-extractable radioactive residues in all four soils were formed in high amounts reaching their maximum of 63.8 to 92.5 % TAR at the end of the study after 120 days of incubation.

C. VOLATILIZATION

Formation of CO_2 was observed in all four soils reaching in total 9.1 to 21.2 % TAR after 120 days. No other volatile compounds were detected.

Table 7.2/3 Distribution of radioactivity in soils after treatment with ^{14}C-bentazone and incubation under aerobic conditions [% TAR] – Bruch West (20°C)

Days after treatment	Extractable				NER	Volatiles*			Material balance
	Methanol	Methanol + water	Acetone	Total		CO_2	Other volatiles	Total	
0	87.5	9.6	0.1	97.2	2.9	n.d.	n.d.	n.d.	100.1
0	87.5	9.5	0.0	97.0	2.9	n.d.	n.d.	n.d.	99.9
0 mean	87.5	9.6	0.1	97.1	2.9	n.d.	n.d.	n.d.	100.0
3	81.2	9.2	0.2	90.6	9.8	0.0	0.0	0.0	100.4
7	72.3	8.6	0.4	81.2	17.7	0.0	0.0	0.0	98.9
14	62.4	7.9	0.1	70.5	27.5	1.4	0.0	1.4	99.4
29	44.8	7.6	0.1	52.5	41.3	5.7	0.0	5.7	99.5
62	21.1	6.5	0.3	27.8	58.2	13.0	0.0	13.0	98.9
62	21.0	6.4	0.3	27.6	58.2	13.9	0.0	13.9	99.8
62 mean	21.0	6.4	0.3	27.7	58.2	13.5	0.0	13.5	99.3
90	8.6	4.3	0.1	13.1	62.8	17.9	0.0	17.9	93.8
120	3.0	3.8	0.1	6.9	69.2	21.2	0.0	21.2	97.4
120	3.1	4.1	0.1	7.3	67.8	21.2	0.0	21.2	96.3
120 mean	3.1	4.0	0.1	7.1	68.5	21.2	0.0	21.2	96.8

n.d. = not determined
NER = non-extractable residues
TAR = total applied radioactivity (100% = 1.949 mg/kg dry weight)
*cumulated values

Table 7.2/4 Distribution of radioactivity in soils after treatment with ^{14}C-bentazone and incubation under aerobic conditions [% TAR] –Li10 (20°C)

Days after treatment	Extractable				NER	Volatiles*			Material balance
	Methanol	Methanol + water	Acetone	Total		CO_2	Other volatiles	Total	
0	90.8	7.6	0.0	98.5	2.0	n.d.	n.d.	n.d.	100.4
0	90.1	6.9	0.1	97.0	2.5	n.d.	n.d.	n.d.	99.6
0 mean	90.4	7.3	0.1	97.8	2.2	n.d.	n.d.	n.d.	100.0
3	88.3	5.2	0.1	93.6	6.4	0.0	0.0	0.0	100.0
7	79.9	5.8	0.3	86.0	11.5	0.0	0.0	0.0	97.5
14	69.4	6.5	0.1	76.0	22.8	0.6	0.0	0.6	99.4
29	53.2	7.1	0.1	60.4	29.2	2.5	0.0	2.5	92.1
62	28.0	6.1	0.2	34.2	63.0	6.5	0.0	6.5	103.7
62	30.1	6.5	0.2	36.8	58.0	6.8	0.0	6.8	101.5
62 mean	29.1	6.3	0.2	35.5	60.5	6.6	0.0	6.7	102.6
90	18.6	5.5	0.1	24.2	61.0	10.2	0.0	10.2	95.4
120	10.0	5.3	0.3	15.6	67.4	13.5	0.0	13.5	96.5
120	10.9	4.0	0.1	15.0	63.8	13.5	0.0	13.5	92.3
120 mean	10.4	4.6	0.2	15.3	65.6	13.5	0.0	13.5	94.4

n.d. = not determined
NER = non-extractable residues
TAR = total applied radioactivity (100% = 1.958 mg/kg dry weight)
*cumulated values

Table 7.2/5 Distribution of radioactivity in soils after treatment with ^{14}C-bentazone and incubation under aerobic conditions [% TAR] –LUFA 2.2 (20°C)

Days after treatment	Extractable				NER	Volatiles*			Material balance
	Methanol	Methanol + water	Acetone	Total		CO_2	Other volatiles	Total	
0	84.7	9.1	0.1	93.8	2.8	n.d.	n.d.	n.d.	96.6
0	91.3	9.6	0.1	100.9	2.4	n.d.	n.d.	n.d.	103.4
0 mean	88.0	9.4	0.1	97.4	2.6	n.d.	n.d.	n.d.	100.0
3	73.3	9.0	0.3	82.6	14.6	0.0	0.0	0.0	97.2
7	69.1	8.4	0.1	77.6	18.2	0.0	0.0	0.0	95.9
14	54.7	8.3	0.2	63.2	35.8	0.6	0.0	0.6	99.6
29	45.0	8.9	0.5	54.4	39.9	2.6	0.0	2.6	96.9
62	14.3	6.0	0.3	20.6	67.5	4.7	0.0	4.7	92.8
62	13.1	5.9	0.3	19.3	67.0	5.5	0.0	5.5	91.8
62 mean	13.7	5.9	0.3	19.9	67.2	5.1	0.0	5.1	92.3
90	8.3	4.8	0.2	13.4	81.8	7.4	0.0	7.4	102.6
120	6.5	4.9	0.3	11.7	92.5	9.1	0.0	9.1	113.2
120	6.3	4.6	0.3	11.3	90.0	9.1	0.0	9.1	110.4
120 mean	6.4	4.8	0.3	11.5	91.3	9.1	0.0	9.1	111.8

n.d. = not determined
NER = non-extractable residues
TAR = total applied radioactivity (100% = 1.891 mg/kg dry weight)
*cumulated values

Table 7.2/6 Distribution of radioactivity in soils after treatment with ^{14}C-bentazone and incubation under aerobic conditions [% TAR] –LUFA 2.3 (20°C)

Days after treatment	Extractable				NER	Volatiles*			Material balance
	Methanol	Methanol + water	Acetone	Total		CO_2	Other volatiles	Total	
0	86.9	9.3	0.2	96.4	2.5	n.d.	n.d.	n.d.	98.9
0	89.6	8.7	0.4	98.6	2.5	n.d.	n.d.	n.d.	101.1
0 mean	88.2	9.0	0.3	97.5	2.5	n.d.	n.d.	n.d.	100.0
3	81.9	7.8	0.3	90.0	8.1	0.0	0.0	0.0	98.1
7	76.5	8.1	0.2	84.8	12.5	0.0	0.0	0.0	97.3
14	65.8	8.2	0.2	74.1	21.2	0.7	0.0	0.7	96.0
29	51.9	9.5	0.8	62.2	31.2	3.2	0.0	3.2	96.6
62	34.8	8.9	0.7	44.3	49.6	8.9	0.0	8.9	102.9
62	29.8	7.5	0.3	37.5	51.0	7.8	0.0	7.8	96.2
62 mean	32.3	8.2	0.5	40.9	50.3	8.3	0.0	8.4	99.5
90	19.1	6.4	0.2	25.8	58.0	12.6	0.0	12.6	96.3
120	13.1	6.1	0.2	19.4	62.8	16.0	0.0	16.0	98.2
120	11.7	5.1	0.1	16.9	67.7	16.0	0.0	16.0	100.6
120 mean	12.4	5.6	0.2	18.1	65.2	16.0	0.0	16.0	99.4

n.d. = not determined
NER = non-extractable residues
TAR = total applied radioactivity (100% = 1.954 mg/kg dry weight)
*cumulated values

D. TRANSFORMATION OF PARENT COMPOUND

All combined methanol and methanol / water extracts were analysed by radio-HPLC. The results are summarized in Table 7.2/7 to Table 7.2/10.

The peak pattern obtained by chromatographic analysis was very similar for all four soils incubations. The concentration of the parent compound (BAS 351 H) decreased in the four soils from 92.6 % TAR at 0 DAT to approximately 4.8-18.8 % TAR after 120 days of incubation. The identification of the parent was achieved by chromatographic comparison with the retention time of the ^{14}C-test item.

The metabolite N-methyl-bentazone (M351H009) was detected in three soils, however only in small amounts showing maximum values of 2.2 (Li 10), 5.4 (LUFA 2.2) and 0.5 (LUFA 2.3) % TAR.

During the course of the study, two minor unknown metabolites were visible in the chromatograms showing maximum values of 2.2 (UK2) and 1.5 (UK1) % TAR.

Table 7.2/7 Radio-HPLC analysis of soil extracts after treatment of soil Bruch West with ^{14}C-bentazone and incubation under aerobic conditions at 20°C [%TAR]

Days after treatment	Total	UK1	Bentazone (BAS 351 H)	N-methyl-bentazone (M351H009)	UK2
		t_R 30.9	t_R 37.9	t_R 47.6	t_R 51.6
0	97.2	0.7	96.5	-	-
0	97.0	1.0	96.1	-	-
0 mean	97.1	0.8	96.3	-	-
3	90.6	0.4	90.2	-	-
7	81.2	0.7	80.5	-	-
14	70.5	1.2	69.2	-	-
29	52.5	0.9	51.6	-	-
62	27.8	-	25.9	-	1.9
62	27.6	-	27.6	-	-
62 mean	27.7	-	26.8	-	0.9
90	13.1	-	13.1	-	-
120	6.9	-	6.9	-	-
120	7.3	-	7.3	-	-
120 mean	7.1	-	7.1	-	-

t_R = retention time [min]
- = not detected
TAR = total applied radioactivity (100% = 1.949 mg/kg dry weight)

Table 7.2/8 Radio-HPLC analysis of soil extracts after treatment of soil Li 10 with ^{14}C-bentazone and incubation under aerobic conditions at 20°C [%TAR]

Days after treatment	Total	UK1	Bentazone (BAS 351 H)	N-methyl-bentazone (M351H009)	UK2
		t_R 30.9	t_R 37.9	t_R 9.5	t_R 51.6
0	98.5	0.5	98.0	-	-
0	97.0	-	96.8	-	0.2
0 mean	97.8	0.2	97.4	-	0.1
3	93.6	1.0	92.6	-	-
7	86.0	-	85.8	-	0.2
14	76.0	1.5	74.5	-	-
29	60.4	1.4	59.1	-	-
62	34.2	-	34.2	-	-
62	36.8	-	36.0	0.8	-
62 mean	35.5	-	35.1	0.4	-
90	24.2	-	21.4	2.3	0.5
120	15.6	-	13.4	2.2	-
120	15.0	-	13.3	1.7	-
120 mean	15.3	-	13.3	2.0	-

t_R = retention time [min]
- = not detected
TAR = total applied radioactivity (100% = 1.958 mg/kg dry weight)

Table 7.2/9 Radio-HPLC analysis of soil extracts after treatment of soil LUFA 2.2 with ^{14}C-bentazone and incubation under aerobic conditions at 20°C [%TAR]

Days after treatment	Total	UK1	Bentazone (BAS 351 H)	N-methyl-bentazone (M351H009)	UK2
		t_R 30.9	t_R 37.9	t_R 9.5	t_R 51.6
0	93.8	1.2	92.6	-	-
0	100.9	1.0	100.0	-	-
0 mean	97.4	1.1	96.3	-	-
3	82.6	0.6	81.9	-	-
7	77.6	0.4	77.0	-	0.3
14	63.2	0.5	62.7	-	-
29	54.4	-	50.5	1.8	2.2
62	20.6	-	17.1	3.5	-
62	19.3	-	16.1	3.2	-
62 mean	19.9	-	16.6	3.3	-
90	13.4	-	8.7	4.7	-
120	11.7	-	6.7	4.1	0.8
120	11.3	-	4.8	5.4	1.0
120 mean	11.5	-	5.8	4.8	0.9

t_R = retention time [min]
- = not detected
TAR = total applied radioactivity (100% = 1.891 mg/kg dry weight)

Table 7.2/10 Radio-HPLC analysis of soil extracts after treatment of soil LUFA 2.3 with ^{14}C-bentazone and incubation under aerobic conditions at 20°C [%TAR]

Days after treatment	Total	UK1	Bentazone (BAS 351 H)	N-methyl-bentazone (M351H009)	UK2
		t_R 30.9	t_R 37.9	t_R 9.5	t_R 51.6
0	96.4	-	95.0	-	1.4
0	98.6	-	96.6	-	2.0
0 mean	97.5	-	95.8	-	1.7
3	90.0	0.7	89.3	-	-
7	84.8	1.0	83.8	-	-
14	74.1	0.5	73.6	-	-
29	62.2	1.3	59.7	-	1.3
62	44.3	-	44.3	-	-
62	37.5	-	37.0	0.3	0.2
62 mean	40.9	-	40.6	0.2	0.1
90	25.8	0.3	25.2	0.2	-
120	19.4	-	18.8	0.5	-
120	16.9	-	16.6	0.3	-
120 mean	18.1	-	17.7	0.4	-

t_R = retention time [min]
- = not detected
TAR = total applied radioactivity (100% = 1.954 mg/kg dry weight)

Calculation of the degradation rates

An overview on the dissipation times of bentazone calculated with the different kinetics is given in Table 7.2/11 and Table 7.2/12.

The χ^2 error level was in all cases lower than 15 %, indicating accurate statistical description by the applied kinetic models. The SFO model showed a better fit for the Bruch West and the LUFA 2.2 soil whereas the FOMC fit was slightly better for the Li10 and LUFA 2.3 fit. However, the fitted parameters were not significant on a 5 % level for the FOMC fit of LUFA 2.3.

For all fits, the excellent quality of the fit was confirmed by visual inspection. The residuals are distributed randomly. Due to the very low χ^2 error level and the higher significance of the SFO kinetic parameters, the SFO kinetics can be regarded as persistence endpoints as well as modelling endpoints in all cases with the exception of the Li 10 soil where the FOMC fit should be used to obtain persistence endpoints.

Table 7.2/11 DT_{50}/DT_{90} values for bentazone in various soils at 20°C calculated with the SFO model

Soil	DT_{50} [days]	DT_{90} [days]	Type I error rate (Prob.>t)	χ^2 error level	Visual fit
Bruch West	**33.0**	**109.6**	**9.1×10^{-13}**	**1.77**	**excellent**
Li10	43.4	144.2	5.4×10^{-13}	1.38	excellent
LUFA 2.2	**30.9**	**102.7**	**2.5×10^{-7}**	**6.65**	**excellent**
LUFA 2.3	**49.1**	**163.2**	**4.9×10^{-10}**	**2.52**	**excellent**

Table 7.2/12 DT_{50}/DT_{90} values for bentazone in various soils at 20°C calculated with the FOMC model

Soil	DT_{50} [days]	DT_{90} [days]	χ^2 error level	α and β at 5% error level	Visual fit
Bruch West	32.1	112.6	2.00	not significant	excellent
Li10	**42.0**	**152.7**	**0.88**	**significant**	**excellent**
LUFA 2.2	27.4	119.0	6.78	not significant	excellent
LUFA 2.3	46.0	188.9	2.36	not significant	excellent

best fit values printed in bold

The fitted curves for the single first-order (SFO) and the first-order multiple compartments (FOMC) are shown in the figure below.

Figure 7.2/ 1 ^{14}C-bentazone: modelled against observed residue

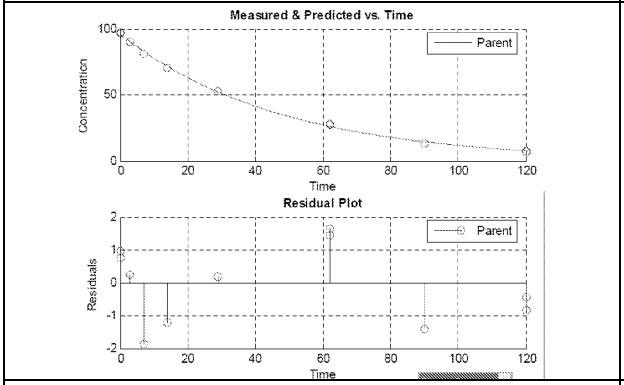
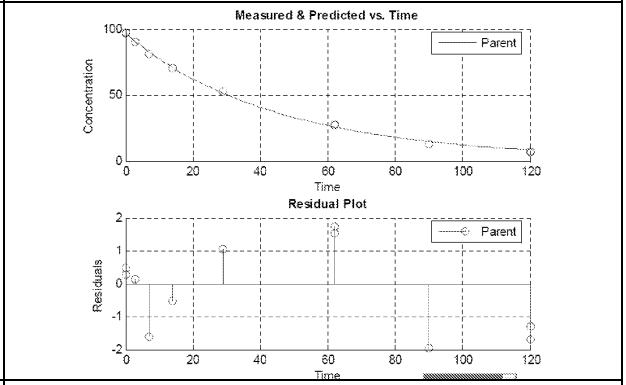
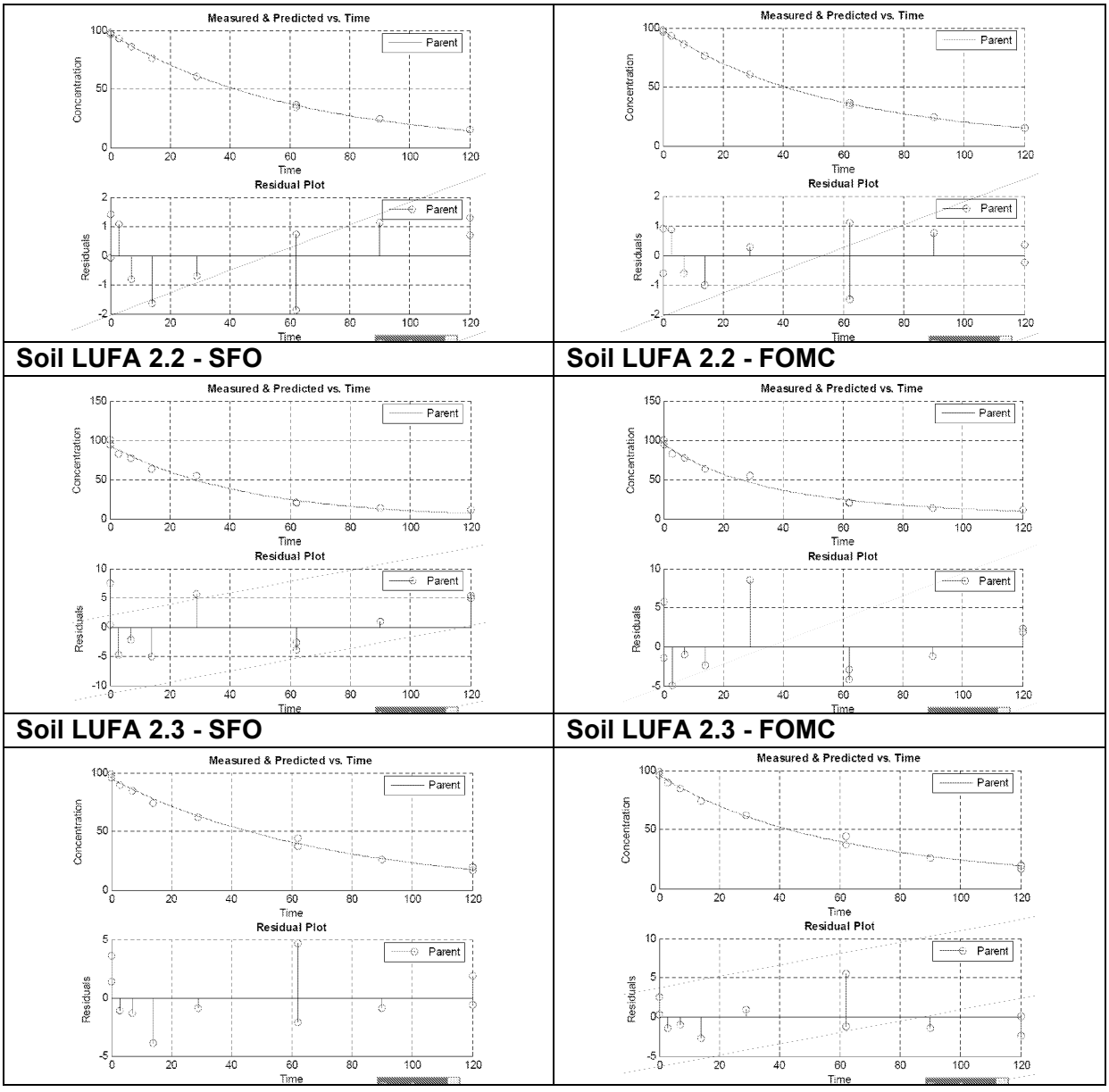
Bruch West - SFO	**Bruch West - FOMC**
	
Soil Li 10 - SFO	**Soil Li 10 - FOMC**

Figure 7.2/ 1 ^{14}C-bentazone: modelled against observed residue



III. CONCLUSION

The results of the present study show that bentazone (BAS 351 H) was degraded in four different soils with SFO half-lives of 33.0 (Bruch West), 43.4 (Li 10), 30.9 (LUFA 2.2) and 49.1 days (LUFA 2.3) when incubated under aerobic conditions at 20°C and a soil moisture of 40 % of the maximum water holding capacity. The calculated SFO values are suitable as persistence endpoints as well as modelling endpoints in all cases with the exception of the Li10 soil where persistence endpoints should be derived from the FOMC model.

In three soils, the known soil metabolite N-methyl-bentazone was detected at a maximum amount of 5.4 % TAR (LUFA 2.2). Two minor unknown metabolites appeared during the course of the study; however, none of them exceeded 2.2 % of the total applied radioactivity (TAR). After 120 days of incubation, the mineralization rate had reached 9.1 - 21.2% TAR and the non-extractable residues amounted to 65.2 - 91.3% TAR.

Report:	II A 7.2.1/2 Li K.-B. et al. 2008(b) Degradation of herbicides Atrazine and Bentazone applied alone and in combination in soils BASF DocID 2008/1098933
Guidelines:	None
Testing Laboratory and dates:	18-Jan-2008
GLP:	No, not subject to GLP regulations (scientific publication)

Executive Summary

The rates of degradation of atrazine and bentazone separately as well as in combination were investigated in rhizosphere soil and non-rhizosphere soil originating from a corn field at Huajiachi Campus, Zhejiang University, Zhejiang Province, China.

The experiments were performed at a temperature of 25 °C in the dark. Soil samples were sieved to 2 mm before use.

Soils were treated with the herbicides at a concentration of 10 mg/kg soil (dry weight) which is considered to be 10 times the maximum recommended application rate used by the farmers.

Duplicate samples were taken 2, 4, 6, 13, 21, 26, 36 and 47 days after treatment (DAT) from the rhizosphere and the non-rhizosphere soil. The autoclaved rhizosphere soil was sampled at 0.7, 3, 4, 6, 9, 12, 15, 17, 20, 26, and 30 DAT. Collected samples were immediately frozen and stored at -20 °C until analysis.

For analysis the soil was extracted three times with a methanol / water mixture (9:1, v/v). The combined extracts were concentrated by rotary evaporation at 45°C to the aqueous phase. The aqueous extracts were three times partitioned against dichloromethane. The combined dichloromethane phase was concentrated to dryness, re-dissolved in a methanol / water mixture (66:44) and analysed by HPLC.

No degradation of either atrazine or bentazone was observed in autoclaved rhizosphere soil, whereas in all experiments using non-autoclaved soil a continuous decrease of the herbicides was found. This indicates degradation of both substances by biotic processes.

The resulting degradation curves were fitted to the first-order model $\ln(c_t/c_0) = -k\ (t - t_{lag})$.

When comparing rhizosphere and non-rhizosphere soil for separately applied herbicides, a faster degradation was found for rhizosphere soil in case of atrazine but also a small lag-phase, which was not observed in non-rhizosphere soil. For bentazone, the degradation rate for rhizosphere and non-rhizosphere soil was comparable; although a lag-phase (6.8 days) was found in non-rhizosphere soil.

Application of a combination of both herbicides led to a diminished degradation rate for bentazone and an elongated lag-phase for both herbicides. This effect was lessened by addition of Tween 20 to the combination.

Although the authors postulated a significant difference in degradation rates of bentazone when applied separately or in combination with atrazine, the calculated degradation rates overall were very similar. Irrespective of the kind of incubation, the half-lives of bentazone ranged between 20 and 25 days.

I. MATERIAL AND METHODS (from Li et al. 2008)

A. MATERIALS

1. Test Material

Bentazone (purity ≥ 97%) was obtained from BASF (Ludwigshafen, Germany). Atrazine (purity ≥ 99%) was purchased from Chemical Service (West Chester, USA). A nonionic surfactant, Tween-20 (chemically pure), was purchased from the Mingqing Chemical Reagent Plant (Wenzhou, China).

2. Soils

Rhizosphere soils (RS) and non-rhizosphere soils (NRS) were collected from the agricultural field at Huajiachi Campus, Zhejiang University, Zhejiang Province, China. The sampling site was planted with corn for at least three consecutive years with no application of a combined formulation of atrazine and bentazone. High-performance liquid chromatography (HPLC) analysis of the soil extracts prior to the start of the study showed no detectable residue of bentazone and atrazine.

At harvest, rhizospheres containing as much as possible associated roots, were excavated by digging to a 20 cm depth around maize plants. Non-rhizosphere soil samples were taken from the same site containing few roots. The rhizosphere, non-rhizosphere soil, and plant samples were placed in plastic bags, immediately transported to the laboratory and prepared within a few hours. Soil loosely adhering to the roots was removed by mild shaking. The remaining adherent soil, called rhizosphere soil, was manually separated and collected in a bucket. Soil samples were sieved to 2 mm and then stored at 4 °C for no more than 14 d prior to initiation of the study. A part of the soil samples was air-dried for 2 d at room temperature for property analysis.

The soil characteristics are summarized in Table 7.2/13.

Table 7.2/13 Properties of soil used to investigate degradation of atrazine and bentazone

Soil designation	Huajiachi Campus
Origin	Zhejiang University, Zhejiang Province, China
Particle size distribution [g/kg] sand silt clay textural class	 5.3 753 241.8 silty clay loam
Organic matter [g/kg]	36.4
pH	6.1

B. STUDY DESIGN

1. Experimental conditions

The degradation of atrazine and bentazone alone was investigated in RS and NRS. Furthermore, the degradation of both herbicides applied to RS as a mixture and as a mixture with Tween 20 was investigated in RS.

The soils were treated at a nominal concentration of 10 mg of each herbicide per kg dry soil, which was equivalent to ten times the recommended application rate of combined herbicide. In case of Tween addition the surfactant was applied at a concentration of 0.27 g/kg soil.

For treatment, appropriate amounts of a methanol application solution were mixed with 25.0 g (dry weight) microbial-inactive soil. After evaporation of methanol and thorough mixing, the spiked portion was incorporated into an aliquot of 1500 g RS (174 g/kg moisture content) or an aliquot of 1500 g NRS (141 g/kg moisture content) at 5.03 g spiked portion per kg soil (dry weight).

The soil moisture of all treatments was adjusted to 290 g/kg with the soil sieved several times to mix thoroughly. The homogenized samples were placed in six 2 L beakers and immediately sampled as day 0. The beakers were then covered with plastic film, which was pierced with a needle to allow air exchange, and incubated in the dark at 25 °C. During the incubation period, the beakers were removed from the incubator every other day and brought to the original moisture content (290 g/kg) by adding the required amount of sterile distilled water.

The degradation of atrazine and bentazone alone in autoclaved RS was also investigated. Fifty grams (dry weight) RS was weighed in 100 mL Erlenmeyer flasks. After being plugged with cotton pads, the flasks were autoclaved at 120 °C for 30 min each day in three consecutive days to remove microbial activity. Atrazine or bentazone 0.5 mg each was added to the autoclaved soil in 1 mL of methanol. Eleven flasks for each herbicide were incubated under the same conditions as described above, except that the spiking was performed in a laminar hood.

2. Sampling

At 2, 4, 6, 13, 21, 26, 36, and 47 days after treatment (DAT), duplicates of about 30 g soil from each treatment (RS and NRS) were sampled and immediately stored at -20 °C until analysis. Treated flasks with autoclaved RS were sampled at 0.7, 3, 4, 6, 9, 12, 15, 17, 20, 26, and 30 DAT and stored at -20 °C.

3. Description of the analytical procedures

At the end of the incubation, the frozen samples were thawed at room temperature. After transferring about 30 g of moist soil to 100 mL Erlenmeyer flasks, soil samples were extracted three times by shaking for 2 h with a 40 mL methanol and water mixture (9:1, v/v) each time. The combined extracts were concentrated in a rotary evaporator below 45 °C under reduced pressure to remove the methanol fraction. The residual aqueous extracts were repartitioned three times with 20 mL dichloromethane. When bentazone was extracted with dichloromethane, the aqueous extract was adjusted to pH < 3.0. The dichloromethane fractions were pooled, passed through anhydrous sodium sulfate and further concentrated to near dryness in a rotary evaporator. Then, N_2 was used to dry the extract and the residue was reconstituted in a 10 mL mobile phase composed of 66:44 methanol:water (pH adjusted to 2.8) for analysis. The HPLC detection limit for bentazone was 5.9 µg/L, and for atrazine, it was 3.8 µg/L.

4. Calculation of degradation rates

A first-order model used was employed to analyse the herbicide degradation kinetics in this study:

$$\ln(c_t/c_0) = -k\,(t - t_{lag}) \qquad (1)$$

$$t_{1/2} = \ln 2/k \qquad (2)$$

where c_t is the concentration of the herbicide at time t, c_0 is the initial concentration of the herbicide, k is the first order degradation rate constant, t_{lag} is the lag phase during which there is no apparent herbicide degradation, and $t_{1/2}$ is the half-life. The parameter estimates were obtained for each degradation condition using linear regression. All experimental data used in the regression analysis were the mean values of duplicates except for those from the degradation of atrazine and bentazone in the autoclaved soil, which were single points without replicates. A t test on k was conducted to detect the differences in the effect of the incubation conditions on the pesticide degradation rates.

II. RESULTS AND DISCUSSION

For calculated degradation parameters see Table 7.2/14.

Atrazine was degraded in both rhizosphere soil (RS) and non-rhizosphere soil (NRS), but not in autoclaved rhizosphere soil, indicating that the degradation was a biotic process.

Furthermore, a significant difference of the degradation rate between RS and NRS soils pointing towards a rhizosphere effect in degradation. Because of a higher amount of organic compounds in rhizosphere soil (root exudates) a higher microbial activity would be expected here, which was confirmed by the higher degradation rate in RS soil.

Bentazone was also degraded in both RS and NRS, but not in autoclaved RS, indicating that degradation was again a biotic process. A lag-phase of 6.8 days was observed in NRS, which was not found in RS. Overall, the degradation rate was not significantly different between both soils.

When atrazine and bentazone were applied in combination to RS, a longer lag-phase and higher half-lives resulted for both compounds compared to single applied herbicides.

For bentazone, the addition of Tween 20 to the herbicide combination resulted in significantly higher degradation rates and a shortened lag-phase compared to atrazine + bentazone without the surfactant.

Table 7.2/14 Degradation parameters of atrazine and bentazone

Herbicide	Treatment	K [d^{-1}]	$t_{1/2}$ [d]	t_{lag} [d]	r^2
Atrazine	Rhizosphere soil	0.02694 (0.00047)[1]a [2]	25.7	2.0	0.99
	Non-rhizosphere soil	0.02386 (0.00213) b	29.1	0.0	0.97
	Rhizosphere soil plus bentazone	0.02249 (0.00171) c	30.8	6.7	0.99
	Rhizosphere soil plus bentazone + Tween 20	0.02255 (0.00339) c	30.7	3.2	0.95
Bentazone	Rhizosphere soil	0.03432 (0.00272) a	20.2	0.0	0.98
	Non-rhizosphere soil	0.03475 (0.00568) a	19.9	6.8	0.95
	Rhizosphere soil plus atrazine	0.02744 (0.00368) b	25.2	6.6	0.97
	Rhizosphere soil plus atrazine + Tween 20	0.03233 (0.00430) a	21.4	4.2	0.96

Degradation parameters are derived from the model $\ln(c_t / c_0) = k(t - t_{lag})$ and $t_{1/2} = \ln 2/k$
[1] Values in parentheses are standard deviation.
[2] Means for a given herbicide followed by the same letter are not significantly different (P = 0.05) using t-test.

III. CONCLUSION

Degradation rates of atrazine and bentazone were compared in Chinese rhizosphere and non-rhizosphere soils for separately applied herbicides atrazine and bentazone as well as for combined applications. For atrazine a higher degradation rate was found in rhizosphere soil, whereas the degradation rate of bentazone was comparable in both soils although with a longer lag-phase in non-rhizosphere soil.

The authors postulated that combination of both herbicides resulted in a slower degradation rate and a longer lag-phase for bentazone and in a longer lag-phase for atrazine compared to the single applied herbicides.

Nevertheless, all calculated degradation rates were very similar. Irrespective of the kind of incubation, the half-lives of bentazone ranged between 20 and 25 days.

Report:	II A 7.2.1/3 Rodriguez Cruz M. S. et al. 2008(b) Study of the spatial variation of the biodegradation rate of the herbicide Bentazone with soil depth using contrasting incubation methods BASF DocID 2008/1098934
Guidelines:	None
Testing Laboratory and dates:	02-Sep-2008
GLP:	No, not subject to GLP regulations (scientific publication)

Information on the degradation rate of bentazone in soil is also provided in this public literature. It describes the differences in degradation rates of bentazone in various soil depths, as well as in samples of the same soil but incubated under different conditions (undisturbed vs. sieved). Although the experiments described in this publication do not follow common guidelines used for pesticide registration and were not performed according to GLP rules, they are considered informative for degradation in deeper soil layers.

Executive Summary

To study the spatial variability of bentazone degradation with soil depth, two experiments were conducted using soil from Long Close field on the farm at Warwick HRI, Wellesbourne, Warwickshire, UK.

For investigation of vertical variability in degradation, soil from five depths (0 - 10, 20 - 30, 40 - 50, 60 - 70, 70 - 80 cm) and three sampling points within the trial field was sampled, sieved to <3mm and treated with Basagran (containing 87% bentazone) to reach a concentration of 5 mg a.s. / kg soil. Samples were adjusted to 40% MWHC (ca.15.5% moisture content) and incubated at 15°C in the dark. Samples were taken in regular intervals within a period of three months and analysed for bentazone. Organic matter content, pH, biomass, and dehydrogenase activity and K_d were determined for all soil samples.

For investigation of vertical and horizontal variability in degradation of bentazone, soil was sampled at two depths (0 - 10 and 50 - 60 cm) at ten different sampling points within the trial plot. Samples were taken as disturbed and undisturbed soil cores. The soil from disturbed soil cores was sieved to <3mm, adjusted to 40% MWHC (ca. 15.5% moisture content) and treated with Basagran as described above. Undisturbed soil cores were treated at the same nominal concentration by injections into the soil core and incubated vertically. Moisture content in the undisturbed soil cores was not standardized across the cores but was found to be 13.3% and 16.7% at harvest in cores from 0 - 10 and 50 - 60 cm, respectively.

All samples prepared in this second experiment were analysed for bentazone after 127 d of incubation at 15°C in the dark.

In the first experiment the degradation of bentazone was found to follow first order kinetics in the top soils (0 - 10, 20 - 30 cm). In the subsoils (30 - 40, 50 - 60, 70 - 80 cm) the degradation was fitted to a linear model. DT_{50} values ranged from 56 to 515 days and were found to increase with soil depth. Furthermore DT_{50} values were found to correlate with organic matter content, pH, biomass, dehydrogenase activity and K_d.

In the second experiment, a faster degradation was found in the intact soil cores for the 0 - 10 cm depth compared to the sieved soil, reflected in the lower amount of bentazone remaining in the soil of intact cores (6.63%) compared to sieved soil (17.2%). In the subsoil (50 - 60 cm depth), no significant difference was found between the intact cores and sieved soil.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

Bentazone as Basagran (87% w/w) was supplied by BASF (Ludwigshafen, Germany). Analytical grade bentazone was supplied by Chem Service Inc. (West Chester, USA).

2. Soil

Soil was sampled in Long Close field on the farm at Warwick HRI, Wellesbourne, Warwickshire, UK. The soil was a sandy loam where bentazone had not been applied in the previous 10 years.

Soil for testing the vertical variability in degradation rate was collected from five depths (0 - 10, 20 - 30, 40 - 50, 60 - 70, 70 - 80 cm) at three sampling locations in the field. After sampling, the soil was spread onto clean polythene bags and left on the bench overnight to reduce moisture content before passing it through surface sterilised sieves (<3 mm).

Furthermore, soil was sampled for testing vertical and horizontal spatial variability in intact soil cores and in sieved soil. This soil was collected from two depths (0 - 10 cm and 50 - 60 cm) at ten different sampling locations in Long Close field.

Soil parameters as total organic matter, microbial biomass, dehydrogenase activity, pH, clay, sand and silt content were measured in pre-sieved soil.

B. STUDY DESIGN

1. Experimental conditions

The pre-sieved soils were treated at a nominal concentration of 5 mg bentazone per kg soil, which is equivalent to concentrations found in the top 0.75cm of soil following application of the maximum recommended dose of 2.5 kg / ha. The test item was applied as commercial bentazone formulation dissolved in distilled water. Further water was added to adjust the soil to about 40% MWHC (-33 kPa) corresponding to a moisture content of ca. 15.5%. The treated soils were thoroughly mixed by hand and further by passing through a <3 mm sieve five times.

Each soil was transferred into a sterile, loosely capped polypropylene container and incubated at 15°C in the dark. Moisture content was maintained by the addition of sterile distilled water as necessary (usually once each week).

The intact soil cores were treated with bentazone by injecting four 250 µL aliquots of commercial formulation into the core to reach a final concentration of 5 mg/kg soil. Two injections were made into the top and two into the bottom of the core, with the "*injection points at each side made 2 cm apart, and at 2 cm depth within the core"*.

The average amount of soil in the cores was 263 g, and at harvest average moisture content in cores was found to be 13.3% and 16.7% in cores from 0 to 10 and 50 to 60 cm depth, respectively, so that at the point of collection from the field moisture content averaged 12.8% and 16.2% in cores from 0 to 10 and 50 to 60 cm, respectively. No attempt was made to standardise water content across the cores, but moisture content was corrected as necessary by the addition of distilled water to the surface of the base and top of the core, so that moisture content of the cores largely reflected that in the field at time of collection. The soil cores were sealed at base and top with parafilm and incubated vertically at 15 °C in the dark.

2. Pesticide extraction and analysis

The pre-sieved soils were sampled at regular intervals over three-month period, extracted and then analysed by HPLC. For the second experiment, the cores were sampled after 127 d incubation. Soil was pushed from the cores, mixed by hand and sieved (<3 mm) five times. Sub-samples (10 g) of each soil were dried in an oven at 110 °C overnight to determine the soil moisture content. Pesticide was extracted from each soil sample and analysed using the procedures indicated above.

Using pre-sieved soil, sorption of bentazone was determined using a batch mixing method, and adsorption distribution coefficients (K_d) measured.

3. Statistical analysis

The best estimate of time 50% degradation (DT_{50}) was derived using the best fitting model, recommended by FOCUS (2006). Data were fitted to a range of different linear or exponential models. The standard errors for DT_{50} values from various models were compared in order to choose the best fit DT_{50} value. Visual inspection was used to confirm that selected models provided acceptable description of the data. The model with the lowest standard error and best visual assessment was considered to be the best fit model. The exponential and linear models were found to provide best fit to the degradation kinetics, and were used to obtain DT_{50} values.

Analysis of variance was used to determine the significance of differences in pesticide degradation between soil depth and method (sieved soils or soil cores). For the study of vertical and horizontal spatial variability in degradation using intact cores and sieved soil, variability within samples was estimated by replicating the measurement of the cores for each treatment combination measured. Due to the fact that the design was unbalanced a general linear model was used for analysis. An accumulated analysis of variance was calculated with the replication of cores giving an estimate of the underlying (within sample) variability for both the core and sieved samples. Predictions were then made for the treatment combinations to give the estimated means and standard errors for each of the treatment combinations tested. Coefficient of variation (%) in the study of vertical and horizontal spatial variability in degradation using intact cores and sieved soil was determined following angular transformation to normalize the variance.

II. RESULTS AND DISCUSSION

Vertical variability in degradation rate using sieved soil

Bentazone decreased with time in all soil depths investigated. As bentazone has a low vapor pressure and a very low sorption in soil, it was concluded that the dissipation of the substance reflects degradation. The degradation curves were found to fit first-order degradation kinetics for the investigated topsoils (0 - 10 and 20 - 30 cm). The degradation in subsoil (40 - 50, 60 - 70 and 70 - 80 cm) was fitted to linear models. DT_{50} values were calculated ranging from 56 to 515 days (see Table 7.2/14). Significant correlations were found between DT_{50} values and soil biomass (r= -0.701, $p < 0.01$), dehydrogenase activity ($r = -0.595$, $p < 0.05$), pH ($r = 0.597$, $p < 0.05$), organic matter content ($r = -0.744$, $p < 0.01$) and K_d ($r = -0.676$, $p < 0.05$).

Vertical and horizontal spatial variability in degradation using intact cores and sieved soil

Generally, degradation was faster in the topsoil than in the subsoil. Furthermore significant differences were found for degradation of bentazone within sieved soil and intact soil cores in the topsoil but not in the subsoil (see Table 7.2/16). Thus in top soil a faster degradation was found in intact soil cores than in sieved soil.

The authors discuss various factors influencing degradation in intact soil cores and in sieved soil:
- the fact that microorganisms are concentrated at the surface of soil aggregates in intact cores limiting contact to the pesticide and conversely higher contact between microorganism and the pesticide in sieved soil
- the likelihood that the pesticide has more contact to sorption sites in sieved soil which might lead to reduced degradation
- the capacity of degraders to spread through soil more rapidly in sieved soil enhancing degradation
- a higher variability of soil moisture within an intact soil core than in sieved soil leading to spatial variability within the cores

No degradation products of bentazone were found with the HPLC conditions used.

Table 7.2/15 Change in key soil properties and bentazone degradation with soil depth

Soil depth (cm)	OM (%)	pH[a]	Biomass [mg C kg^{-1} soil][a]	Dehydrogenase [µg TPF g^{-1} soil][a]	DT_{50} [d][b]
0 - 10	2.62	7.06	65.5	43.1	56
20 - 30	2.33	7.13	64.5	22.9	65
40 - 50	1.98	7.79	36.4	10.7	178
60 - 70	1.66	7.98	21.5	7.48	306
70 - 80	1.35	8.09	19.1	6.60	515
Least significant difference (p>0.05)	0.29	0.45	16.0	14.7	178
Significance of effect of depth	***	**	***	***	***

Data represents mean of three sampling locations at each depth.
TPF Triphenyl formazan.
NS, not significant.
**Significant, p<0.01.
***Significant, p< 0.001
[a] From Bending and Rodríguez-Cruz (2007).
[b] obtained from sieved soil.

Table 7.2/16 Key soil characteristics and percentage of bentazone remaining after 127 d incubation in sieved soil and intact cores

Soil depth (cm)	OM (%)	pH[a]	Biomass [mg C kg^{-1} soil][a]	Dehydrogenase [µg TPF g^{-1} soil][a]	Bentazone remaining in sieved soil [a b] [%]	Bentazone remaining in intact cores[a][%]
0 - 10	2.71	6.71	445	43.2	17.2 (23.7)	6.63 (12.5)
50 - 60	1.43	7.30	23.4	3.87	60.1 (51.4)	64.7(54.21)
Least significant difference (P>0.05)	0.47	0.53	16.3	11.9	3.0	
Significance of effect of depth	***	***	***	***	***	***

TPF Triphenyl formazan.
[a] Data in brackets gives angular transformed data, to which LSD relates, for comparison between sieved soil and cores, and for the effect of depth.
[b] Data subset from those reported in Rodríguez Cruz et al. (2006).
***Significant, p<0.001.

III. CONCLUSION

Degradation of bentazone showed significant vertical and horizontal variability. The rate of degradation slowed down with increasing depth. DT_{50} values ranged from 56 in top soil to 515 days in 70 - 80 cm depths when incubated at 15°C. Significant correlations were found with biomass, dehydrogenase content, organic matter content, pH and K_d.

Furthermore, differences were found in degradation behaviour between intact soil cores and sieved soil. In intact soil cores, only about 7% of the initial applied bentazone could be detected after 127 days, whereas in sieved soil still 17% was left.

7.2.2 Aerobic degradation of the active substance in soils at 10°C

Aerobic degradation half-lives at 10 °C can be derived by extrapolation from degradation half-lives obtained from e.g. 20°C-data using a Q10-value of 2.58 as proposed by EFSA, 2007 (Opinion on the default Q10 value).

Therefore the half-lives of the degradation rate study with bentazone described in Section II A 7.2.1/1 (Tornisielo & Sacchi, BASF DocID 2011/1000621) were extrapolated to 10 °C according to the following equation.

$$f_{temp} = Q_{10}^{\left(\frac{Temp_{act} - Temp_{ref}}{10}\right)}$$

where:
f_{temp} = temperature normalisation factor
Q10 = factor of change of the degradation rate at temperature change of 10°C (2.58)
$Temp_{ref}$ = reference temperature to which the degradation rate is scaled [here: 10 °C]
$Temp_{act}$ = temperature at which the study was carried out [here: 20 °C]

The extrapolated half-lives are shown in Table 7.2/17. The resulting DegT50 values scaled to 10 °C range from 79.7 to 126.7 days with a geometric mean value of 99.0 days. The DegT90 values scaled to 10 °C range from 264.8 to 420.8 days with a geometric mean value of 329.0 day.

Table 7.2/17 Laboratory half-lives and DegT90-values of bentazone at 10 °C

Soil	DegT50 original [days]	DegT90 original [days]	Temperature normalization factor - f_{temp} [-]	DegT50 normalized to 10 °C [days]	DegT90 normalized to 10 °C [days]	Source
Bruch West	33.0	109.6	2.58	85.1	282.8	II A 7.2.1/1 #2011/1000621
Li10	43.4	144.2	2.58	112.0	372.0	
LUFA 2.2	30.9	102.6	2.58	79.7	264.8	
LUFA 2.3	49.1	163.1	2.58	126.7	420.8	
Geometric mean	**38.4**	**127.5**		**99.0**	**329.0**	

Report:	II A 7.2.2/1 Smelt J.H. 2003(b) Laboratory studies on the degradation rates of Bentazone in Dutch soil profiles BASF DocID 2002/1011918
Guidelines:	Discussienotitie ten aanzien van de omzetting van bestrijdingsmiddelen in de waterverzadigde ondergrond - Richtlijnen voor het onderzoek
Testing Laboratory and dates:	BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 01-Oct-1999 - 31-Jan-2002
GLP:	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

The dissipation of BAS 351 H - bentazone in soil systems was investigated in freshly collected Dutch soils from three depths (topsoil, unsaturated subsoil, saturated subsoil) of fields in five relevant agricultural regions. The fields were carefully selected considering soil properties, planted crops and bentazone use in the respective areas. Also hydrogeological information was included.

For collection of the subsoils, special core sampling equipment was used and care was taken to ensure no contamination with surface soil. Saturated subsoils of those sites which showed more anaerobic conditions were transported and stored under Argon gas atmosphere.

Subsamples of approximately 100 g (dry weight) of the topsoil layers and non-saturated subsoil layers were incubated in the dark at a temperature of 15 °C, and of the saturated layers at 10 °C. After a pre-incubation time of 5 - 7 days, bentazone was applied, giving an initial content of 1 mg/kg dry soil in topsoil and 0.01 or 0.02 mg/kg in the unsaturated subsoils.

For the saturated subsoils, subsamples of average 170-200 g (dry weight) were incubated under saturated conditions with 48-59 mL groundwater at a temperature of 10 °C in closed serum bottles with either a nitrogen atmosphere in the headspace (for three anaerobic subsoils) or in not airtight closed bottles with atmospheric condition (for two aerobic subsoils). After a pre-incubation time of 5 - 7 days, bentazone was applied, giving an initial content of 0.005 mg/kg dry soil. Redox potential and pH were monitored over the study duration. No clear changes were measured in either the aerobic saturated subsoils or the anaerobic saturated subsoils.

Duplicate samples were analysed at increasing time intervals, for a maximum duration of 462 days. Sampling were adapted to the expected or observed rates of decrease of bentazone.

The results showed that degradation of bentazone (at 15°C) could be observed in the topsoils as well as in the unsaturated subsoils of all five locations. The DT_{50} in topsoils was approximately two weeks for four locations, and four weeks for the fifth location. The DT_{50} in unsaturated subsoils was between approximately one week and eight weeks for four locations, and 32 weeks for the fifth location.

In saturated subsoils (incubated at 10°C), bentazone degradation was slowed down considerably, as the remaining fraction of bentazone was still between 54.9 % and 92.9 % at the end of the study.

I. MATERIALS AND METHODS

1. Test material

BAS code:	BAS 351 H
Common name:	Bentazone
Reg. No.:	51929
CAS-No.:	25057-89-0
Chemical name (IUPAC):	3-isopropyl-1H-2,1,3,-benzothiadiazin-4(3H)-one-2,2-dioxide
Molecular weight:	240.28 g/mol

2. Test soils

Five fields from five typical agricultural regions in the Pleistocene area of the Netherlands with high (potential) use of bentazone were selected. No bentazone had been applied to any of the selected fields in at least the previous 4 years.

From each field, soil samples from three soil depths (top layer, unsaturated subsoil layer, water-saturated subsoil) were collected. Soil from the top layer (0 - 25 cm) and the unsaturated subsoil layer (40 - 90 cm) were sampled with an auger, while the water-saturated subsoil (2.2 - 4.4 m depth depending on groundwater table) was sampled using a special core sampling tube. The saturated subsoil sampling was performed under conditions limiting the contact of air by using nitrogen gas (aerobic subsoils) or argon gas (anaerobic subsoils) in the sampling tube during the coring process. For transport, samples were transferred to flasks without a headspace.

Storage of top layer and unsaturated subsoil material was at 15 °C ± 3 °C, in the dark, for 10 to 25 days before starting the incubation experiment. Storage of saturated subsoil material was at 10 °C ± 3 °C for a maximum period of 1 to 10 days before application of bentazone.

Undisturbed soil was collected for determination of water content at the desired pF value for incubation of the unsaturated soils. Oxygen concentration and redox potential were measured in the sampling holes at the selected fields.

The site characteristics of the sampled soils are presented in Table 7.2/18.

Table 7.2/18 Soil characteristics of the sampled soils

Location	**Vredepeel**			**Vlagtwedde**			**Buurse**		
Soil class (Dutch specification)	podzol			podzol			dikke enkeerdgrond		
Depth (cm)	0-25	50-80	220-310 *	0-25	50-80	235-320 *	0-25	50-80	310-400
Clay (< 2 µm) %	2.7	1.9	2.1	2.4	2.4	2.6	2.1	3.2	2.3
Silt (2-50 µm) %	7.8	0.1	0.6	10.4	2.9	1.2	5.8	7.0	0.0
Sand (50-2000 µm) %	84.7	97.5	97.2	81.3	94.4	96.1	88.4	84.1	97.6
Organic Carbon (g/100 g dry soil)	2.89	0.31	0.04	5.52	0.15	0.04	2.43	3.73	0.03
$CaCO_3$ (g/100g dry soil)	0.1	< 0.1	< 0.1	0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
pH(H_2O)	6.4	5.3	5.5	5.6	5.3	5.6	5.8	4.8	5.2
pH(KCl)	6.2	4.5	4.5	4.9	4.4	4.4	5.0	4.0	4.6
CEC ($cmol^+$/kg dry soil)	8.9	0.3	< 0.1	8.6	< 0.1	< 0.1	4.9	4.1	< 0.1
Microbial biomass (mg C/100 g dry soil)	15.6	-	-	18.1	-	-	13.6	2.3	-
Saturated subsoil data §									
Temperature (°C)	7.9			7.4 - 8.1			8.0		
pH	4.9 – 5.07			5.06 - 5.5			4.37 – 4.45		
Oxygen (mg/L)	0.48 – 0.91			0.40 - 0.71			8.9 – 9.6		
Redox potential (mV) **	263 - 344			231 - 251			437 - 515		

Table 7.2/18 Soil characteristics of the sampled soils

Location	**Esbeek**			**Roosendaal**		
Soil class (Dutch specification)	dikke enkeerdgrond			podzol		
Depth (cm)	0-25	40-70	320-420	0-25	60-90	320-410 *
Clay (< 2 µm) %	3.1	2.8	2.3	3.1	4.0	3.7
Silt (2-50 µm) %	15.4	15.6	0.8	6.2	1.2	0.3
Sand (50-2000 µm) %	78.1	79.9	96.9	86.8	94.1	95.8
Organic Carbon (g/100 g dry soil)	2.15	1.09	0.02	2.24	0.24	0.04
$CaCO_3$ (g/100g dry soil)	< 0.1	< 0.1	< 0.1	< 0.1	0.1	0.1
pH(H_2O)	5.8	5.5	5.5	6.1	6.2	5.0
pH(KCl)	4.8	4.6	4.5	5.4	5.1	4.3
CEC ($cmol^+$/kg dry soil)	2.6	0.8	< 0.1	5.3	0.4	0.7
Microbial biomass (mg C/100 g dry soil)	20.8	1.8	-	25.8	-	-
Saturated subsoil data §						
Temperature (°C)	9.8 – 9.9			9.2 – 10.2		
pH	4.57 – 5.06			4.19 – 4.45		
Oxygen (mg/L)	6.0 – 7.1			0.53 – 0.76		
Redox potential (mV) **	408 - 492			270 – 323		

§ range of 2-3 measurements at the date of collection, *in situ* measurements
- not measured
* anaerobic conditions in saturated soil layer
** corrected to standard hydrogen electrode

3. Experimental treatments

Pre-incubation
Subsamples of the top soil layers and unsaturated subsoil layers (corresponding to 100 g dry weight) were incubated in in 250 mL bottles under dark conditions at a temperature of 15 °C. The saturated soil samples were incubated at 10 °C. During preparation of the saturated soil subsamples, anaerobic conditions were maintained by flushing the equipment with nitrogen or argon gas as during field sampling. After a pre-incubation time of 5 to 7 days, bentazone was applied to the soils.

Application and sample preparation for unsaturated soils
All flasks with top sojls (0 - 25 cm layer) were dosed with 1 mL of a solution containing 100 µg/mL bentazone in HPLC water, giving a concentration of about 1 mg/kg dry soil. This corresponds to a field application rate of about 1.3 kg a.s./ha, assuming an equal distribution over the top 5 cm and a soil density of 1.5 kg/dm. The unsaturated subsoils (between 40 – 90 cm depth) of the fields Vlagtwedde, Vredepeel and Roosendaal were dosed with 1 mL of a solution containing 1.0 µg/mL bentazone in HPLC water, giving a concentration of about 0.01 mg/kg dry soil. The unsaturated subsoils of Buurse and Esbeek were dosed with 1 mL solution containing 2.0 µg/mL bentazone. The higher dose was chosen to reduce possible problems with interfering peaks with analyzing low remaining fractions. The 1 mL solutions were applied with a 1 mL syringe, which was emptied drop-wise onto the soil surface.

The desired moisture content for each unsaturated soil during incubation was determined by measuring the water content of undisturbed soil cores in equilibrium with a hanging water column of 150 cm (pF-value of 2.17) for the 0 - 25 cm layer and a 70 cm water column (pF-value of 1.84) for the samples from the layers collected between 40 - 90 cm depth, in triplicate.

The unsaturated soil samples were stored at 15°C ± 3°C in dark cabinets as during pre-incubation. Gas exchange was possible through small holes in the caps. Water contents were monitored and kept constant by keeping the flasks in an almost water-saturated atmosphere.

Application and sample preparation for saturated soils
The saturated subsoils were incubated at a temperature of 10 °C in closed serum bottles with either a nitrogen atmosphere in the headspace (for three anaerobic subsoils) or in not airtight closed bottles with atmospheric condition (for two aerobic subsoils, oxygen containing). The soil mass in each incubation ranged from 170 to 200 g (average dry weight), and the water mass ranged from 48 to 59 g.

Using a syringe, 1.0 µg bentazone was applied to the water phase in each serum bottle. The dose of 1 µg resulted in a concentration in the water phase of about 20 µg/L or 5 µg/kg dry soil.

The bottles were closed again with the screw caps with rubber septa (not airtight for aerobic saturated subsoils, airtight for anaerobic saturated subsoils) and placed in dark temperature cabinets at 10°C ± 3°C. Anaerobic samples were handled with an additional N_2 flow to minimize oxygen contact.

4. Sampling and analysis

At each sampling point, two flasks for each soil layer were extracted. Scheduled sampling intervals for the topsoils (0 - 25 cm) were at 1, 7, 14, 21, 28, 35, 49, 63, 77, 105 and 133 days after addition of bentazone. Scheduled sampling intervals for the unsaturated subsoils (40 - 90 cm) were at 1, 7, 14, 21, 28, 42, 56, 84, 112, 168, 224 and 280 days after treatment, while for the saturated subsoils (220 - 420 cm), scheduled sampling intervals were at 1, 14, 28, 56, 84, 112, 140, 196, 252, 308, 364 and 420 days after treatment.

Samplings were not continued if less than 10 % of the initially applied dose remained. The decrease in the water-saturated subsoils was followed over approximately 460 days, and redox potential and pH were monitored over the study duration.

Topsoil samples were extracted by adding 100 mL methanol to the 250 mL Schott flasks. The suspension was shaken during 1 h on a reciprocating shaker (200 min^{-1}) with the flasks in a horizontal position. Part of the slurry was decanted in centrifuge tubes and the closed tubes were centrifuged until the organic layer was clear. Part of the extract (about 4 mL) was transferred to HPLC vials and stored in a refrigerator until analysis with HPLC. Control samples of about 100 g were included and extracted with the same method.

The incubated soil portions from the unsaturated subsoils were extracted by adding 100 mL 0.01 M $CaCl_2$ solution. The soil portions of the water-saturated layers contained already a lot of water, therefore only 50 mL 0.01 M $CaCl_2$ solution was added. The closed flasks were shaken (horizontal position) on a reciprocating shaker (200 min^{-1}) for 1 h and the slurry was decanted into centrifuge tubes and centrifuged (2000 min^{-1}) to clear the liquid layers. The extracts were acidified with HCl, and bentazone was then extracted with CH_2Cl_2. After evaporation of the extraction solution, the dried residue was re-dissolved in 2 mL methanol and analysed for bentazone.

Two unsaturated subsoils had relatively high organic matter contents (>0.5% organic C; Buurse and Esbeek). For good extraction efficiency, these soils were extracted with methanol as described for the topsoils, but including a concentration step to achieve sufficiently low detection limits.

5. Method validation

Specimens were analysed for bentazone by HPLC. The method was validated within the study as follows: during the incubation experiment, in each analytical series at least one soil type was spiked with bentazone at the level of application at the start of the incubations (100 µg for the subsoils and 1.0 µg for the two subsoils) and a lower level near the limit of quantification (2 µg for the topsoils and 0.1 µg for the subsoils). The origin of the field of the spiked soil batches was changed with the analysis series in a way that all fields were at least once used for the recovery tests during the experiment. At each analysis time, soil samples of the analysed depths were spiked with the two levels of bentazone. Not spiked soil portions were used as blanks. Soil portions to be spiked were prepared at the start of the incubations, for each of the three sampling depths of all five fields.

For each analytical series a calibration curve was prepared with at least 4 (generally 5) concentrations levels of bentazone in the appropriate concentration range. Duplicate injections were done of a lower number of concentration if concentrations in the analysis series were in a narrow range.

II. RESULTS AND DISCUSSION

A. Method validation

Results from the recovery tests for the high doses indicated that recoveries were high (79.8 – 105.8 % of applied), except for the unsaturated subsoils extracted with 0.01 M $CaCl_2$, where one sample had an inexplicably low recovery of 39 %. However, the extracted unsaturated subsoils of the incubation series (including one day after application) had high recoveries in that analytical series, so the 39 % in the validation must be seen as an exception.

Recoveries for the low doses were between 69.1 and 165.6 %. A relatively high standard deviation in the unsaturated subsoil with MeOH extraction and the water-saturated subsoils was observed and is mainly caused by one sample in each series. However, the blank sample showed also a peak at the retention window of bentazone at that time. Corrections for this would deliver reasonable extraction efficiencies. These observations for the low concentration range do not affect the results of the incubations, because the amount of bentazone in the water-saturated subsoils hardly decreased during the incubation experiment.

B. Findings

Monitoring of redox potential and pH over the study duration indicated that conditions were within the ranges measured in the field, and no clear changes were measured with incubation time in either the aerobic subsoils or the anaerobic subsoils.

Topsoil samples

The amounts of bentazone recovered in the five incubated topsoils (0 - 25 cm layers) at the sampling times are shown in Table 7.2/19. Decrease in the masses of bentazone was regular in time with small differences between the duplicates at each sampling time in all five soils. The decrease was quite similar and fast (DT_{50} about 2 weeks) in the Vlagtwedde, Roosendaal and Buurse soil. The Vredepeel soil had the slowest decrease with a DT_{50} of about 4 weeks.

Table 7.2/19 Recovered amounts of bentazone (% of applied) in topsoils (0-25 cm). Soils incubated at 15 °C.

Time (days)	Vredepeel		Vlagtwedde		Buurse		Esbeek		Roosendaal	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
1	93.5	93.2	91.3	92.7	81.3	73.7	91.4	89.5	80.1	77.9
7	80.3	80.1	64.4	64	59.9	54.6	68.4	67.6	56.4	59.7
14	72.4	68.4	42.5	43.1	49.2	48.4	55.9	54	49.6	49.3
21	62.3	60.5	30.7	30.4	33.8	31.6	42	52	37.2	35.7
28	53.1	53.5	22.4	22.5	25.6	31.2	35.1	37.6	24.6	26
35	44.7	45.7	16.3	15	31.8	21.6	28.1	24.2	16.9	19.1
49	34.6	35.2	10.9	8.8	10.4	9.9	15.4	20.6	8.2	9.3
63	19.9	21.1	3.6	3	7.4	5	12.5	14.8	6	7.6
77	16.7	16.7	2.6	2.8			6.5	9.4		
105	9.8	11.3								

Unsaturated subsoil samples

The results for the unsaturated subsoils, collected between 40 and 90 cm depth, are shown in Table 7.2/20. Esbeek and Buurse soil showed a fast decrease in bentazone concentration. Degradation was almost identical or even faster (Buurse) than in the topsoil of the respective location. However, the much lower but representative concentration in the subsoil must be taken into account (1 µg per 100 g against 100 µg in topsoils). The fast degradation might be related to the relatively high organic carbon contents which are typical for fields with a long history of agricultural use and organic manuring.

The Vredepeel and Roosendaal soils also had a distinct and regular decrease in bentazone concentration with incubation time. The Vlagtwedde soil showed the slowest decrease with incubation time, although continuous.

Table 7.2/20 Recovered amounts of bentazone (% of applied) from the unsaturated layers. Soils incubated at 15 °C.

Time (days)	Vredepeel (50 – 80 cm)		Vlagtwedde (50 – 80 cm)		Buurse (50 – 80 cm)		Esbeek (40 – 70 cm)		Roosendaal (60 – 90 cm)	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
1	97.1	95.1	97.2	89.3	80.3	81.3	95.9	96.6	92.4	94.4
7	83.2	81.9	87.9	75	50.4	53.2	81.2	72.2	85.4	87.5
14	61.6	64.8	82.4	83.4	32.2	31.2	61.8	64.9	71.8	78.4
21	73	81.2	92.8	95.3	16.6	19.1	46.6	51.4	76.3	72.7
28	62.1	67.2	85.6	90.3	10.5	11.3	37.7	35.8	74	77.5
35	54.6	54.6	87	89.1						
42	48.9	51.4	80.6	88.6			27.7	25.1	62.9	64.1
56	36.6	28.9	88.2	82.2	5.5	5.0	15.7	22.7	55.1	56.5
84	16.3	13.5	79.5	82			9.3	10.4	45	43
112	8.4	5.3	76.8	76					31.8	31.4
168	0	0	64.9	63.5					16.2	15.2
224			52.2	52.6					5.5	5.6
280			41.3	41						

Saturated subsoil samples

The results for the water-saturated subsoils are shown in Table 7.2/21. Bentazone appeared to be quite stable in the samples from the upper part of the permanently saturated subsoils (depth depending on lowest groundwater table) during the long time period of the incubation experiment (462 days). However, a tendency of decrease was observed in the Buurse soil and an initial decrease in the Vlagtwedde soil was observed, but did not continue.

Table 7.2/21 Recovered amounts of bentazone (% of applied) from the water-saturated layers. Soils incubated at 10 °C.

Time * (aerobic / anaerobic)	Vredepeel (anaerobic)		Vlagtwedde (anaerobic)		Roosendaal (anaerobic)		Buurse (aerobic)		Esbeek (aerobic)	
	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2	Rep. 1	Rep. 2
1 / 1	104.8	99.5	94	90.6	105.8	106.8	112.9	114.1	102.3	99.9
13 / 14	100	104	100.1	91.1	102.6	108.8	103.7	101.8	124.7	103.5
27 / 28	102.8	95.4	96.6	100.1	106.9	110.9	105.3	122.7	101.3	109
55 / 56	98.9	96.4	84.7	82.5	92.4	91.8	88.6	95.6	92.3	94.3
83 / 84	98	99.3	79.7	97.8	102.3	104.9	91.6	90	84.2	101.3
111 / 112	91.8	100.2	77	75.2	100.4	88	95.3	81.5	97.7	80.7
141 / 142	94.4	87.2	61.7	68.9	101.3	106.3	79.9	76.1	89.5	87.9
195 / 196	82.3	88.6	47.3	67.8	97.1	99.6	82.2	81.1	868	72.5
251 / 252	91.8	90.5	57.2	63.7	106	86.3	79.5	84.2	86	91
307 / 308	98	83.6	55.8	69.4	107.1	102.4	80	83.9	89.9	91.3
364 / 365	103.4	92.6	69.1	59.6	89.8	89.6	72.8	81.7	79.4	92.7
461 / 462	88.9	92.9	66.8	76.4	61.1	79.7	57.8	54.9	80.6	84.1

* sampling times in days after application, slightly different for anaerobic and aerobic soils

III. CONCLUSION

The dissipation of bentazone in soil systems was investigated in five Dutch soils from three depths (topsoil, unsaturated subsoil, saturated subsoil) of fields in relevant agricultural regions.

Degradation of bentazone (at 15°C) was observed in the topsoils and unsaturated subsoils of all five locations. The DT_{50} in topsoils was approximately two weeks for four locations, and four weeks for the fifth location. The DT_{50} in unsaturated subsoils was between approximately one week and eight weeks for four locations, and 32 weeks for the fifth location.

In saturated subsoils (incubated at 10°C), bentazone degradation was slowed down considerably as the remaining fraction of bentazone was between 54.9 % and up to 92.9 % at the end of the study.

Report: II A 7.2.2/2
Veen J.R. van de 2003(b)
Kinetic evaluation of the degradation rates of Bentazone in 5 soil profiles
BASF DocID 2002/1008886

Guidelines:

Testing Laboratory and dates: BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 28-Aug-2003 - 28-Aug-2003

GLP: No, not subject to GLP regulations

Since the degradation rates mentioned in the above summarized study (*Smelt*, 2003) were only rough graphical estimations from the degradation curves, an extra kinetic evaluation was performed by J.R. van de Veen (2003(b)). The summary is given below.

Executive Summary

The dissipation of bentazone in different soil layers was evaluated by applying a 1st order kinetic model to the data generated in Smelt (2003). ModelMaker 3.0.4 was used to implement a single first-order (SFO) compartmental model, and the resulting half-lives were converted to half-lives at a reference temperature of 20 °C, using a Q_{10} value of 2.2.

The resulting degradation parameters and DT_{50} values confirmed rapid degradation of bentazone in the topsoil with half-lives (at reference temperature 20 °C) of 8.8-21.6 days, and in the unsaturated subsoil with half-lives of 6.4 - 48.2 days (and one value of 196 days, where degradation was slow but with a continuous decrease). However, in the water-saturated subsoil the calculated degradation rates were much longer showing values between 270 and 1380 days.

MATERIAL AND METHODS

Input data for the modelling study

Soils were designated by their sampling locations (Vredepeel, Vlagtwedde, Roosendaal, Buurse and Esbeek) and depth (topsoil, unsaturated subsoils, saturated subsoils). A summary of the experiments is given in the study above [Smelt (2003)], where the experimental data can be found. Replicates of the sampling points were not averaged, but used individually as modelling input.

Estimation of the degradation rate constants

The data for topsoils, unsaturated subsoils and saturated subsoils were evaluated by implementing a single first-order (SFO) compartmental model in ModelMaker 3.0.4. Only one transition step from bentazone to a sink compartment (summarizing all losses) was considered. The definition of the transition steps between bentazone and sink is shown in Equation 7.2/ 1.

Equation 7.2/ 1 Model definition in ModelMaker 3.0.4

$$\frac{d\ Bentazone\_in\_soil}{dt} = -F_{12} = -k_{12} \cdot Bentazone\_in\_soil$$

$$\frac{d\ SINK}{dt} = +F_{12} = +k_{12} \cdot Bentazone\_in\_soil$$

with:	Bentazone_in_soil	compartment 1 representing the concentration time course of bentazone in the specific soil layer
	SINK	compartment 2 representing elimination of bentazone from the system by degradation (no experimental data assigned)
	F_{12}	transition from compartment i to compartment j
	k_{12}	1st order rate constant for transition from compartment 1 to compartment 2

The 1st order rate constant (k_{12}) was estimated using the non-linear regression algorithm of Marquardt-Levenberg with option Least Squares as implemented in the ModelMaker 3.0.4 package. Initial M_0 and k were set to 100 and 0.01, respectively. A t-test was employed (α = 0.05) to identify the probability that a parameter was not significantly different from zero.

RESULTS AND DISCUSSION

The resulting parameter values, corresponding DT_{50} values and r² of the parameter estimation are summarized in Table 7.2/22. The half-life was converted to a half-life at a reference temperature of 20 °C using the Arrhenius equation and a Q_{10} value of 2.2, and is also given in the table below. The visual fits of the soils were very good for topsoils and unsaturated subsoils, but less so for the saturated subsoils, where degradation was slow.

Table 7.2/22 Half-lives of bentazone in Dutch soil profiles

Location	Depth	k (d^{-1})	r^2	Half-life at experimental temp. condition [d]		Half-life at reference temp. 20°C [d]
Vredepeel	topsoil	0.021655	0.998	32.0	at 15°C	**21.58**
	50-80 cm	0.018395	0.978	37.7	at 15°C	**25.40**
	water saturated *	0.000115	0.993	3036.1	at 10°C	**1380.1**
Vlagtwedde	topsoil	0.053368	0.998	13.0	at 15°C	**8.76**
	50-80 cm	0.0023843	0.990	290.7	at 15°C	**196.00**
	water saturated *	0.0011033	0.965	628.2	at 10°C	**285.6**
Buurse	topsoil	0.037206	0.985	18.6	at 15°C	**12.56**
	50-80 cm	0.073049	0.947	9.5	at 15°C	**6.40**
	water saturated *	0.000312	0.983	595.0	at 10°C	**270.4**
Esbeek	topsoil	0.033156	0.993	20.9	at 15°C	**14.09**
	40-70 cm	0.031523	0.994	22.0	at 15°C	**14.82**
	water saturated *	0.000311	0.982	1288.4	at 10°C	**585.7**
Roosendaal	topsoil	0.040992	0.995	16.9	at 15°C	**11.40**
	60-90 cm	0.0096888	0.995	71.5	at 15°C	**48.23**
	water saturated *	0.00052295	0.984	1325.5	at 10°C	**602.5**

*incubated at 10 °C

CONCLUSION

The dissipation of bentazone in different soil layers was well described by the simple 1st order kinetic model. The high coefficient of determination (r^2= 0.95-0.99), low type-I error rate (<0.001) and good agreement between measured and calculated dissipation curves gave evidence of a good overall fit of the 1st order kinetic model.

The resulting degradation parameters and DT_{50} values confirmed rapid dissipation of bentazone with half-lives (at reference temperature 20 °C) in the soil phase of the topsoil of 8.8-21.6 days, in the unsaturated subsoil of 6.4 - 48.2 days (and one value of 196 days, where degradation was slow but with a continuous decrease), and in the water-saturated subsoil of 270 - 1380 days.

Report: II A 7.2.2/3
Leistra M. et al. 2001(b)
Rate of Bentazone transformation in four layers of a humic sandy soil profile with fluctuating water table
BASF DocID 2001/1021063
Guidelines: None
Testing Laboratory and dates: 11-Jun-2001
GLP: No, not subject to GLP regulations (scientific publication)

Executive Summary

The transformation of bentazone was followed in samples from four layers and three collection sites of a humic sandy soil from an experimental field near Vredepeel (Netherlands).

The investigated soil layers included the humic sandy plough layer (0 - 0.25 m, layer A, two dates), the sandy vadose zone with fluctuating water table (0.5 - 0.75 m, layer B, four dates and 1.0 - 1.2 m, layer C, four dates) and the permanently water-saturated sandy subsoil (2.0 - 2.5 m, layer D, one date). Soil samples were collected as indicated at up to four dates within a seven-month period and transferred to the laboratory.

Incubation samples for the layers A to C were generated by mixing equal quantities of soil from the three collection sites X, Y and Z. For layer D samples from the different sites were incubated separately.

For each incubation, 39 g were weighed in for layers A, B and C and 98 g for layer D and treated with bentazone at contents between 0.0109 and 1.32 µg/g dry soil. The soil was incubated at the constant temperature of 15°C in the dark at similar moisture conditions as found in the field at sampling. Aliquots of the water-saturated subsoil were incubated under anaerobic conditions. Furthermore, to check biological influence, selected soil samples were gamma-sterilized and treated with bentazone as well.

Samples were incubated for periods between 84 and 278 days. At distinct sampling times two soil batches of the incubation series for layers A to C were extracted with water and the further processed extract analysed for bentazone by HPLC. Single batches were extracted for the layer D series for each of the collection sites X, Y and Z.

The first-order transformation rates determined varied between the different soil layers, with the date of soil collection and with the initial content of bentazone applied.

The highest transformation rates were found in layer A, followed by the water-saturated layer D and then by layers B and C. In general, the lower the applied concentration of the test item the higher were the transformation rates found. For the sterilized soil, transformation was remarkably slowed down (Layer A and D) or not measurable (Layers B and C) suggesting that transformation is mainly a microbial process.

Additionally, the physico-chemical characteristics of the soil (texture, pH, $CaCO_3$, organic carbon, dissolved organic carbon, nitrogen and water extractable phosphorous) as well as the microbial biomass were determined in subsamples of the collected soil.

Transformation rates showed the same trend as some of the soil factors i.e. the organic carbon content and the water soluble phosphorous content, although a clear relation was difficult to see.

The nitrate-nitrogen levels did not have a visible effect on transformation.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material

BAS code:	Bentazone
CAS-No.:	25057-89-0
Chemical name (IUPAC):	3-isopropyl-1H-2,1,3,-benzothiadiazin-4(3H)-one-2,2-dioxide
Molecular weight:	240.3 g/mol (unlabeled)
Chemical purity:	99.2%

2. Soils

Four different layers of a humic sandy soil from Vredepeel experimental farm (near the village of Vredepeel (Province of Limburg, The Netherlands) were investigated. The soil type of the field is common in areas where groundwater is extracted for drinking water supply. The field (about 1.5 ha) had been grown with grass from 1993 onwards; the grass sod was roto-tilled in November 1996. Soil batches for the incubation studies were collected on four dates: 23 September 1996, 27 November 1996, 5 February 1997 and 17 April 1997. It was expected that the depth of the water table would fluctuate substantially in this period, from comparatively deep in late summer to shallower in early spring. The depth to the groundwater table was measured (every 48 mm) via two groundwater tubes, with pressure gauge and data logger (Arrow Groundwater Level Logger). During the collection period, water-table depth range between 0.8 and 1.4 m.

The soil was collected at three sites on the field, designated as X, Y and Z. The distance between site X and site Y was 30m, while that between these two sites and site Z was 130 m. The soil batches were taken from four layers, depths of 0 - 0.25 m (layer A; two dates), 0.5 - 0.75 m (layer B; four dates), 1.0 - 1.2 m (layerC; four dates) and the 2.0 - 2.5 m (layer D; one date). The soil sample from the permanently water-saturated layer (2.0 - 2.5 m) was collected using a cylindrical vacuum sampler. This material was collected in a polyethylene bag or vessel, which was topped up with groundwater from the site and then sealed hermetically.

Soil characteristics are shown in Table 7.2/23.

Table 7.2/23 Properties of soil layers used to investigate degradation of bentazone

Layer	A (0-0.25m)	B (0.50-0.75 m)	C (1.0-1.2 m)	D (2.0-2.5 m)
Origin	Vredepeel, The Netherlands	Vredepeel, The Netherlands	Vredepeel, The Netherlands	Vredepeel, The Netherlands
Particle size distribution [%]				
silt 0.002 – 0.050 mm	3.1	0.4	0.4	8.9
clay < 0.002 mm	3.6	2.3	2.2	3.6
textural class	sand	sand	sand	sand
Organic matter [%]	4.8	0.4	0.2	0.4
$CaCO_3$ [%]	0.1	<0.1	<0.1	<0.1
pH [KCl]	5.3	4.5	4.6	4.5
pH [H_2O]	6.5	5.9	5.9	5.1
In soil horizon	A_p	B_e/C_e	C_e	C_f

The redox potential in the four layers was measured in the field on 12 days during the period from September to April. The redox potential in layers A, B and C was about 500 mV, indicating that these layers were aerobic. In the permanently water-saturated layer D, the redox potential was about 200 mV, which indicates moderately anaerobic conditions.

B. STUDY DESIGN

1. Experimental conditions

The incubations were started within two weeks after the collection of the soil materials, which had been stored under conditions approaching those in the corresponding layers in the field for this short period of time. Equal quantities of soil material from the corresponding water-unsaturated layers A, B and C at the collection sites X, Y and Z were combined and mixed. The soil mixtures were dried slightly, so that the same moisture content as that in the field would be obtained after the addition of the bentazone in aqueous solution. The moisture contents of the soils were measured by drying sub-samples to constant mass at 105°C.

After each collection, moist soil (39 g) from a layer was weighed into each of a series of glass centrifuge tubes (90 mL). The tubes were covered with aluminium foil, provided with a small hole for air exchange. They were placed in a rack in a box with a layer of water and loosely covered with a lid to prevent the soils from drying out. The soils were pre-incubated for one week in a constant-temperature cabinet at 15°C in the dark.

Aqueous solutions of bentazone (99.2% pure) were prepared at different concentrations, and 1 mL of solution was applied to the soil in each tube. Using a syringe, the solution was distributed in small drops over the enlarged soil surface in the tilted tube, after which the contents of the tube were mixed by shaking and rolling. For each experiment, 1 mL of solution was applied to the soil in a tube and mixed in. The dose was checked by adding 1 mL of the aqueous solution to dichloromethane after each tenth addition to the soil. The soils were incubated in the dark at 15°C.

The incubation of bentazone in the water-saturated subsoil material from layer D (collected in April) was carried out for each of the collection sites X, Y and Z separately. The water-saturated material (98 g) was transferred from the collection bags or vessels to the centrifuge tubes using a small cylindrical auger that could be switched to flushing with nitrogen gas or to suction by a low-pressure system. The incubation tubes were then flushed with nitrogen and closed air-tight with wetted ground-glass stoppers. The wet subsoil materials were also pre-incubated for 1 week in a constant-temperature cabinet at 15°C in the dark. Subsequently, 1 ml of an aqueous bentazone solution (purged with nitrogen) was added by injection throughout the subsoil material. The space heads of the tubes were flushed again with nitrogen and the tubes were closed with the wetted stoppers. The tubes were incubated at 15°C in the same conditions as those in the pre-incubation period.

The redox potential in the (non-sterilized) material from layer D was measured at intervals during the incubation period (April series). The average redox potential in the incubations for layer D was 300 mV (n=72; SD 60 mV), which was somewhat higher than the potential of around 200 mV measured in layer D in the field.

In some of the series, bentazone was also incubated in gamma-irradiated soil materials. For this purpose, the soil was weighed into serum bottles (250 mL) provided with a rubber septum and a screw cap. The soils were exposed to gamma irradiation at 25 kGy. In the aerobic incubations, the septum was pierced with an injection needle provided with a Millex HV filter (0.45 µm) for the application of the bentazone solution and for air exchange. In the anaerobic incubations, the injection needle plus filter were only used to add the solution.

A summary of the incubation series, with the soil layers and collection times, is given in Table 7.2/24. The soil moisture contents in the incubations corresponded to those in the field at the time of collection. The comparatively high initial bentazone content in layer A material was based on the levels expected to occur in this layer in practice. The much lower initial contents in the materials from layers B and C correspond to the low levels that occur in these layers in the field. The initial bentazone content in the layer D materials had to be increased because background substances interfered with the analysis at very low levels.

Table 7.2/24 Soil moisture contents, initial contents of bentazone (calculated on dry soil basis), incubation period and number of measuring points in the incubation series for the Vredepeel soil layers

Layer	Month of collection	Fresh/ sterilized	Soil moisture content [%]	Initial content of bentazone [µg/g]	Incubation period [days]	Number of measuring points
0 - 25 cm	November 1996	Fresh	18.7	1,32	151	28
(15°C)	April 1997	Fresh	16.6	0.117	130	15
	April 1997	Fresh	16.6	1.16	130	18
	November 1996	Sterilized	18.7	1.32	84	18
50 - 75 cm	September 1996	Fresh	7.0	0.0116	193	26
(15°C)	November 1996	Fresh	117	0.0119	278	28
	February 1997	Fresh	8.4	0.0109	270	30
	April 1997	Fresh	9.6	0.0130	165	20
	April 1997	Fresh	9 6	0.110	195	22
	November 1996	Sterilized	11.7	0.01 19	112	17
1.0 - 1.2 m	September 1996	Fresh	16.6	0.0126	256	28
(15°C)	November 1996	Fresh	Saturated	0.0126	278	22
	February 1997	Fresh	Saturated	0.0114	270	30
	April 1997	Fresh	27.4	0.0150	195	22
	April1997	Fresh	274	0.127	195	21
	November 1996	Sterilized	Saturated	0.0126	112	18
2.0 - 2.5 m	April 1997	Fresh	Saturated	0.0269	165	10
(15°C)	April 1997	Fresh	Saturated	0.0515	165	10
	April 1997	Sterilized	Saturated	0.0354	165	10

2. Sampling

Two soil batches of the incubation series for layers A, B and C were extracted each time, while single soil batches were extracted for the layer D series for each of the sites X, Y and Z.

3. Description of the analytical procedures

Analysis of bentazone

Bentazone was extracted by adding 50 ml of HPLC-grade water to the incubation tubes with soil and shaking mechanically for 1 h. The tubes were then centrifuged and the clear supernatant water layer transferred by pipette to another tube. Hydrochloric acid (37%; 1 mL) and distilled dichloromethane (50 ml) were added to this supernatant, after which the tubes were shaken mechanically for 1 h. The dichloromethane layer was pipetted into a tube which was placed in a water bath at 40°C and the solvent evaporated to a small volume with the help of a nitrogen flow. The dried residue was taken up in methanol + HPLC water (3 + 7 by volume; 1 mL) by repeated ultrasonic vibration. If the liquid was turbid, it was filtered through a Millex-HV filter (0.45 µm). The bentazone concentrations were measured using HPLC.

The extraction recovery, derived from the measurements after 1 day, was 81% (n 8; SD 6%) for layer A, 94% (n = 10; SD 7%) for layer B, 97% (n = 12; SD= 5%) for layer C and 80% (n 8; SD=8%) for layer D.

Microbial biomass
The biomass of bacteria in the soil was measured after the soil materials had been collected in November and April using confocal laser scanning microscopy.

Organic carbon and nutrients
The organic carbon content was measured by igniting a dried soil sample in an oxygen stream at 600°C and measuring the evolved carbon dioxide in an infrared cell. Nitrate-nitrogen was measured by extraction with calcium chloride solution, followed by specific spectrophotometric measurement. Water-extractable phosphorus was determined by extraction with water, followed by spectrophotometry.

Concentrations of dissolved organic carbon (DOC) in soil pore water were measured after three soil collections, in November, February and April. They were measured for separate samples from each of the plots X, Y and Z of the Vredepeel field and from each of the four soil layers A, B, C and D. About 200 cm^3 of moist soil was placed in a centrifuge tube provided with a sieve plus filter paper (8 µm). The tube with its contents was centrifuged at 8000 g for 20 min. The collected pore water was filtered through a cellulose acetate filter (0.45 µm) by applying a vacuum. It was checked that the filters hardly affected DOC concentration in water, as compared to glass filters. The DOC concentrations were measured with a carbon analyser (Dohrmann DC-190). The DOC was calculated by subtracting inorganic carbon from total carbon.

4. Calculation of degradation rates
An attempt was made to describe the transformation of bentazone in the soil materials by first-order kinetics. The rate coefficient was calculated by linear regression of the logarithm of the fraction remaining against time. The percentages of variance accounted for by the regression and the standard error of the rate coefficients were calculated, as well as the corresponding half-lives. Analysis of variance was used to test whether the effect of some of the factors on the rate coefficient of bentazone transformation was significant.

II. RESULTS AND DISCUSSION

A compilation of degradation parameters (degradation rates and half-lives) of all investigated soil samples is given in Table 7.2/25.

For ease of comparison the degradations of all trials were described as first-order kinetics although some of the A layer degradation curves were rather following kinetics with changing degradation constants.

Generally, soil sterilization was remarkably slowing down degradation of bentazone suggesting that the observed bentazone dissipation is basically a microbial process. One exception to this were soil samples from the top layer (A) and the water saturated layer (D), where small transformation rates were still measurable. The authors admit that no sterility check has been taken place and thus a small fraction of the microbial population might have survived (e.g. as resistant spores).

As a general tendency it was found that the degradation rates increased with lower initial bentazone content. The authors discuss as possible interpretations that higher levels of bentazone inhibit microbial activity or a comparatively low density of micro-organisms was able to transform bentazone.

Comparing the four layers of soil tested (eg for the April collection time, where similar application doses were chosen for layer A, B and C), the highest degradation rates were found for the top layer A, followed by B and C. In contrast to earlier investigations of the fate of bentazone in subsoil, degradation was measurable in the current study, which might be due to the incubation temperature of 15°C, which is at the upper part of the range found at 1 m soil depth. The authors emphasize that bentazone degradation shows a remarkably steep dependence on temperature.

Comparatively high degradation rates were also found for soil from the saturated layer D. The authors admit that much longer half-lives (1.5 to 2.0 years) for bentazone are found in the literature for water-saturated sandy subsoils with comparatively high pH and rather high redox potentials. The short half-life (38 days) observed in the current study might be explained by the presence of fossile organic matter in the investigated soil layer which may have stimulated microbial activity. Furthermore, the comparable high incubation temperature of 15°C might have contributed to this result, as lower degradation rates for bentazone were found in water-saturated soil at 10°C in other studies.

The rate of bentazone transformation was also set in relation to organic carbon content, DOC, the water-extractable phosphorous levels, the nitrate-nitrogen levels and the biomass (see also Table 7.2/26). The degradation rate was high in layer A, where also the highest organic carbon content and the highest DOC were found. There was no significant difference between organic carbon content of layer B and D, however, a higher transformation was found in layer D.

In the water-saturated layer D the DOC was similar low, whereas the bentazone transformation was higher than those in layer B and C.

The water-extractable phosphorous content was much higher in layer A than in layer B, C and D. This corresponds to the transformation rate being highest in this layer. The levels in layer B and C were much lower (with no significant difference between them). The transformation rates, however, were lower in layer C than in layer B. The water-extractable phosphorous level in layer D was higher than in layer B and C corresponding to a higher transformation rate in layer D. The nitrate-nitrogen levels did not have a visible effect on transformation.

A high biomass content was found for layer A in November and April. This corresponded to high transformation rates in layer A. However biomass contents below limit of quantification were found in layer B and C and for November in layer D and a relatively low biomass for layer D in April, which does not allow direct correlation to transformation rates.

Table 7.2/25 First-order rate constants and half-lives for the transformation of bentazone in soil material from the four layers of the Vredepeel field. Initial contents on the basis of dry soil

Layer	Month of collection	Fresh/ sterilized	Initial content [µg/g]	Rate constant [days^{-1}]	Standard error [days^{-1}]	Half-life [days]
0 - 25 cm	November 1996	Fresh	1.32	0.0299	0.0007	23.2
	April 1997	Fresh	0117	0.0694	00065	10.0
	April 1997	Fresh	1.16	0.0437	0.0021	15.8
	November 1996	Sterilized	1.32	0.0044	0.0008	156
50 - 75 cm	September 1996	Fresh	0.0116	0.0182	0.0012	38.0
	November 1996	Fresh	0.0119	0.0047	0.0004	147
	February 1997	Fresh	0.0109	0 0067	0.0002	104
	April 1997	Fresh	0.0130	0.0074	0.0007	93.4
	April 1997	Fresh	0.110	0.0030	0.0002	230
	November 1996	Sterilized	0.0119	nd[a]		
1.0 - 1.2 m	September 1996	Fresh	0.0126	0.00279	0.00021	248
	November 1996	Fresh	0.0126	0.00372	0.00040	186
	February 1997	Fresh	0.0114	0.00340	0.00022	204
	April 1997	Fresh	0.0150	0.00224	0.00041	309
	April1997	Fresh	0.127	0.00199	0.00027	349
	November 1996	Sterilized	0.0126	nd[a]		
2.0 - 2.5 m site X	April 1997	Fresh	0.0269	0.0236	0.0025	29.3
			0.0515	0.0195	0.0033	35.6
site Y	April 1997	Fresh	0.0269	0.0191	0.0040	36.4
			0.0515	0.0080	0.0011	86.9
site Z	April 1997	Fresh	0.0269	0.0240	0.0039	28.9
			0.0515	0.0159	0.0030	43.5
site X	April 1997	Sterilized	0.0354	0.00219	0.00117	317
site Y	April 1997	Sterilized	0.0354	0.00268	0.00104	259
site Z	April 1997	Sterilized	0.0354	0.00853	0.00308	81.3

[a] no distinct transformation

Table 7.2/26 Soil nutrient characteristics and microbial biomass possibly related to the rate of bentazone transformation in the layers of the Vredepeel field

	Organic carbon [%]		DOC [mg/L]		Nitrate-nitrogen [mg/kg]		Water-extractable phosphorus [mg/kg]		Biomass [µg C/g moist soil]	
Layer	**Average**	**SD (n)**	**Average**	**SD (n)**	**Average**	**SD (n)**	**Average**	**SD (n)**	**Soil collected November 1996**	**Soil collected April 1997**
0 - 25 cm	2.99 a	0.53 (12)	93.2 d	27.3 (9)	14.7 g	11.6 (12)	22.7 i	3.06 (12)	70	260
50 - 75 cm	0.198 b	0.087 (12)	60.4 e	25.5 (9)	10.4 g	14.1 (12)	1.07 j	0.25 (12)	<10	<10
1.0 - 1.2 m	0.090 c	0.047 (12)	42.1 e	14.4 (9)	13.7 g	11.2 (12)	0.86 j	0.28(12)	<10	<10
2.0 - 2.5 m	0.230 b	0.110 (12)	20.5 f	8.6 (6)	1.05 h	1.05 (12)	2.18 k	0.50 (12)	<10	20

DOC = dissolved organic carbon in the pore water
(n) number of measurements
Nutrients averaged for the three sites in the field and for collection times
Values with different letters in the columns are significantly different according to Student's t-test (α=0.05)

III. CONCLUSION

Overall, the results of the present publication show that the degradation of bentazone in soil decreases with increasing soil depths. Major factor for the degradation rates proved to be microbial activity which depends on various factors like temperature and nutrient availability, and if suitable conditions exist allowing for degradation processes even in deeper subsoils, especially at lower bentazone concentrations which can be assumed at soil depths.

7.2.3 Aerobic degradation of relevant metabolites in soils at 20°C

Report:	II A 7.2.3/1 Class T. 2005(b) Aerobic soil degradation of N-Methyl-Bentazone in three standard soils at 20°C BASF DocID 2005/1026922
Guidelines:	EEC 91/414 Annex II (Part A Section 7.1.1); EEC 95/36; OECD 307
Testing Laboratory and dates:	PTRL Europe GmbH; Ulm; Germany Fed.Rep. 12-May-2004 - 08-Dec-2004
GLP:	Yes (laboratory certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

The aerobic soil degradation of N-methyl-bentazone was investigated in a loamy sand (LUFA Speyer 2.2), a loam (LUFA Speyer 3A) and a clay loam (PTRL soil). The soils were all freshly collected from the field, and sieved through a 2 mm screen. Before treatment, the soil moisture was adjusted to 40-50 % of the maximum water holding capacity. The microbial biomass was determined (Anderson & Domsch) after acclimatization, during and at the end of the incubation.

Individual soil samples consisted of 50 g soil (dry weight equivalents) and placed in open incubation flasks covered only partially to allow air exchange, but to prevent excessive loss of soil humidity. The incubations were kept in the dark at 20 ± 2 °C.

Non-labelled N-methyl-bentazone was dosed at 0.64 mg / kg or 32 µg per 50 g (dry-weight) soil incubation. This dose was calculated assuming a bentazone application rate of 1.5 kg/ha and a resulting metabolite formation of max. 0.32 kg/ha, corresponding to an initial concentration of 0.64 mg/kg dry soil.

Soil samples were taken and analysed for remaining N-methyl-bentazone after the following incubation periods: 0 (in duplicate), 1, 2, 3, 4, 6, 8, 15, 30, 37, 60 (duplicate), 90, 120 (duplicate), 150 (duplicate), and 181 (duplicate) days.

All dosed and incubated soil specimens were extracted, cleaned-up using SPE and analysed right after the respective incubation period, using the ion trap LCQ LC/MS/MS.

At the end of the 181-days incubation period, all stored raw soil extracts and the raw extracts obtained from the 21 concurrent recovery soil samples, were diluted 10-fold and analysed without any further SPE clean-up (thus eliminating any potential losses) using the more sensitive triple-quadrupole API 3000 LC/MS/MS instrument. The purpose of these re-analyses was to confirm the degradation data by eliminating day-to-day variations when using the LCQ instrument over the period of 6 months. The improvement in repeatability is demonstrated by a decrease of the relative standard deviation for the 21 concurrent recoveries from ± 11 % obtained by LCQ, to ±8 % obtained by the API 3000 instrument.

The experimental data set obtained by the API 3000 LC/MS/MS analysis for the aerobic degradation of N-methyl-bentazone in the three different soils at 20 °C was evaluated using single first order (SFO) kinetics. Non-linear regression analysis resulted in DT_{50} values of 38 - 153 days and DT_{90} values of 126 - 508 days.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material

BAS code:	BH 351-N-Me
Reg.No.:	79520
Batch-No.:	2235-09
CAS-No.:	61592-45-8
Chemical name (IUPAC):	3-isopropyl-1-methyl-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide
Molecular weight:	254.31g/mol (unlabeled)
Chemical purity:	99.8%

2. Soil

Three soils were selected for the study: a loamy sand (LUFA Speyer 2.2), a loam (LUFA Speyer 3A) and a clay loam (PTRL soil).

The soils were freshly taken from the field and passed through a 2 mm sieve. The actual water content of the sieved soils as received was determined by drying aliquots overnight at 105 °C in the oven and weighing the loss of water. The sieved soil was then brought to a moisture content of approx. 40 % of its maximum water holding capacity by adding distilled water. Before application, they were acclimatized at room temperature with an adjusted water content for several days.

A summary of the soil characteristics is given in Table 7.2/27.

Table 7.2/27 Properties of soils used to investigate the degradation rate of N-methyl-bentazone under aerobic conditions

Soil designation	LUFA Speyer 2.2 (F221904)	LUFA Speyer 3A (F3A 1804)	PTRL soil
Origin	Hanhofen, Germany, Rhineland-Palatinate	Altlußheim; Baden-Württemberg Germany	Ulm-Ermingen, Baden-Württemberg, Germany
DIN Particle size distribution [%] sand 0.063 – 2 mm silt 0.002 – 0.063 mm clay < 0.002 mm textural class	 76.5 15.6 7.9 slightly loamy sand	 42.4 40.7 17.0 sandy loam	 27.9 44.4 27.7 clayey loam
USDA Particle size distribution [%] sand 0.050 – 2 mm silt 0.002 – 0.050 mm clay < 0.002 mm textural class	 77.5 14.6 7.9 loamy sand	 48.5 34.6 16.9 loam	 30.1 42.2 27.7 clay loam
Organic C [%]	2.29	2.2	1.31
Organic matter [%] *	3.95	3.79	2.26
pH [$CaCl_2$]	5.7	7.1	6.8
Cation exchange capacity [$cmol^+$ / kg]	11	17	15
Maximum water holding capacity [g/100g dry soil]	48.4	51.2	45.5
Microbial biomass (6 days before start of study) [mg C/100g dry soil]	19	30	28
Microbial biomass (after 62 days) [mg C/100g dry soil]	56	94	53
Microbial biomass (after 120 days) [mg C/100g dry soil]	46	89	39

* organic matter = organic carbon x 1.724

B. STUDY DESIGN

1. Experimental conditions

For each soil type, a total of 22 1L glass test vessels each containing 50 g dry soil weight (approximately 60 g of moistened soil corresponding to 40 – 50% of the maximum water holding capacity) were prepared. Before application, the soil was stored for several days at room temperature for acclimatization. The water holding capacity was determined again by drying soil aliquots and the soil was adjusted to moisture contents of approx. 40 % of the maximum water holding capacity.

Non-labelled N-methyl-bentazone was dosed at 0.64 mg / kg or 32 µg per 50 g (dry-weight) soil incubation. This dose was calculated assuming a bentazone application rate of 1.5 kg/ha and a resulting metabolite formation of max. 0.32 kg/ha, corresponding to an initial concentration of 0.64 mg/kg dry soil. The application rate was calculated on the basis of an equal distribution of the test item in the top 5 cm soil layer and a soil density of 1.0 g/cm³.

The application solution with the test item was prepared in water by diluting a stock solution (1.00 mg/mL in methanol) to obtain a nominal concentration of N-methyl-bentazone of 32 µg/mL. For application, the calculated amounts of the treatment solution were applied to each soil sample separately (32 µg test item to a portion of 50 g dry soil) using 1 mL of the application solution.

For incubation, the test vessels were covered to prevent excessive loss of water, but to allow exchange of air. They were kept in a thermostated cabinet set to 20 °C, with the cabinet door approximately 1-2 cm open for air exchange.

2. Sampling

Soil incubations were extracted after the following incubation periods: 0 (in duplicate), 1, 2, 3, 4, 6, 8, 15, 30, 37, 60 (duplicate), 90, 120 (duplicate), 150 (duplicate) and 181 (duplicate) days.

3. Analytical procedures

Prior to soil incubation, the following analytical method for the determination of N-methyl-bentazone in soil was developed and validated.

The soil incubations were extracted with 100 mL of methanol by shaking for 0.5 h. Samples for analysis by the ion trap LCQ LC/MS/MS were centrifuged. A 20 mL aliquot of the raw extract was mixed with 20 mL of water and concentrated on a 200 mg EN Lichrolut SPE cartridge. The retained analyte was eluted from the SPE material with 5 mL of acetone. The acetone eluate was further eluted through a 2 g SCX SPE cartridge. The eluate was concentrated to dryness and the residue dissolved in methanol/water (1/1 v/v) for LC/MS/MS analysis.

The soil analysis method was validated before start of the soil experiment at the limit of quantification (LOQ) of 0.05 mg/kg and at the initial application rate of 0.64 mg/kg, resulting in average recoveries of 102 % (±13 %, n=9) and 98 % (±11 %, n=9), respectively.

Samples intended for analysis by the API 3000 LC/MS/MS were prepared without SPE clean-up by diluting the final extract with methanol and water (1/4/5, v/v/v).

The average recovery over the entire 181-days incubation period determined by processing freshly fortified soil (0.64 mg/kg) was 103 % (±11 %, n=21, using the LCQ LC/MS/MS instrument) and 112 % (±8 %, n=21, using the API 3000 LC/MS/MS instrument, without any clean-up of the raw extracts).

4. Kinetic modelling

Table 7.2/28 lists the residues determined by extraction and API 3000 LC/MS/MS analysis and the recovery in %. Based on these results, kinetic evaluation and estimation of degradation times were done using the appropriate mathematical model to describe the concentration time curves. Results shown in the tables in italics were excluded as outliers from kinetic calculations.

Two different kinetic models were applied to describe the degradation of N-methyl-bentazone in soil: single first-order kinetics (SFO) and first-order multi-compartment kinetics (FOMC, also known as Gustafson-Holden model). The Solver function in Microsoft Excel was used to optimise free parameters, i.e. the initial applied amount M_0 and the degradation rate constant k (SFO model) or the kinetic parameters α and β (FOMC model), respectively.

The goodness-of-fit for the optimised degradation curves was assessed by visual and statistical methods. The minimum error level to pass the χ^2 test was defined as the criterion for the best-fit model, i.e. the model with the lowest error level was selected.

II. RESULTS AND DISCUSSION

The results of HPLC-MS analysis (API 3000 LC/MS/MS) are shown in Table 7.2/28.

Table 7.2/28 Degradation of N-methyl-bentazone in soil incubated under aerobic condition at 20°C

Days after treatment	LUFA Speyer 2.2 (F221904)		LUFA Speyer 3A (F3A 1804)		PTRL soil	
	N-methyl-bentazone [mg/kg]	Recovery [%]	N-methyl-bentazone [mg/kg]	Recovery [%]	N-methyl-bentazone [mg/kg]	Recovery [%]
0 repl. 1	**0.705***	110%	**0.690***	108%	**0.702***	110%
0 repl. 2	**0.727***	114%	**0.692***	108%	**0.719***	112%
1	**0.702**	110%	**0.675**	106%	**0.713**	111%
2	**0.693**	108%	**0.660**	103%	**n.p.**	n.p.
3	**0.656**	102%	**0.640**	100%	**0.693**	108%
4	**0.680**	106%	**0.636**	99%	**0.642**	100%
6	**0.702**	110%	**0.594**	93%	**0.658**	103%
8	**0.675**	106%	**0.570**	89%	**0.616**	96%
15	**0.638**	100%	**0.455**	71%	**0.563**	88%
30	**0.620**	97%	**0.444**	69%	**0.458**	72%
37	**0.572**	89%	**0.442**	69%	**0.433**	68%
60	**0.563**	88%	*0.389*	61%	**0.385**	60%
60	**0.576**	90%	*0.405*	63%	**0.367**	57%
90	**0.451**	70%	**0.068**	11%	*0.052*	8%
120 repl. 1	**0.451**	70%	*0.363*	57%	**0.172**	27%
120 repl. 2	**0.449**	70%	*0.345*	54%	*0.330*	52%
150 repl. 1	**0.323**	51%	**0.020**	3.1%	**0.008**	1%
150 repl. 2	**0.350**	55%	**0.018**	2.8%	**0.009**	1%
181 repl. 1	**0.275**	43%	**0.016**	2.5%	**0.010**	2%
181 repl. 2	**0.297**	46%	**0.017**	2.6%	**0.007**	1%

n.p. not performed
Values in *italics* were defined as outliers for kinetic analysis
R_{dosed} = 0.64 mg/kg
* mean of two values

Kinetic evaluation

The estimated best-fit DT_{50} and DT_{90} values for N-methyl-bentazone in aerobic soils are shown in Table 7.2/29.

Table 7.2/29 Estimated DT_{50} and DT_{90} and χ^2 - error levels

Soil	SFO kinetics results			SFO statistical indices		FOMC statistical indices	
	k [d^{-1}]	DT_{50} [d]	DT_{90} [d]	r^2	χ^2 error [%]	r^2	χ^2 error [%]
LUFA Speyer 2.2 (Loamy Sand)	0.0045	153	508	0.9727	3.2	0.9727	3.3
LUFA Speyer 3A (Loam)	0.0183	38	126	0.9815	7.9	0.9815	8.2
PTRL Soil (Clay Loam)	0.0141	49	164	0.9756	6.9	0.9756	7.2

III. CONCLUSION

The degradation rates of N-methyl-bentazone were investigated in three different soils incubated under aerobic conditions at 20 °C over a period of six months (181 days). Kinetic evaluation of obtained experimental results revealed half-lives 38 - 153 days and DT_{90}-values between 126 to 164 days when applying single first-order (SFO) kinetics.

7.2.4 Anaerobic degradation of the active substance in soil

A new anaerobic soil metabolism study was performed with bentazone since last Annex I listing. The study is described in detail in this dossier in chapter AII 7.1.2. The following degradation rate results were obtained:

Table 7.2/30 DT_{50}/DT_{90} values for bentazone incubated in soil first under aerobic and then under anaerobic conditions

Incubation phase	Kinetic model	DT_{50} [d]	DT_{90} [d]	Chi^2
Aerobic	SFO	16.0	53.1	2.2
Aerobic	FOMC	18.5	283	1.4
Anaerobic	SFO	>1000	>1000	2.3

The results show that bentazone is stable in soil under anaerobic conditions.

7.2.5 Anaerobic degradation of relevant metabolites in soil

No study on the anaerobic degradation of a bentazone metabolite was performed. The only soil metabolite detected in amounts approaching 5% of the applied radioactivity was N-methyl-bentazone. This metabolite was detected also in trace amounts (~0.3% AR) in the anaerobic soil metabolism study with the parent bentazone (see chapter AII 7.1.2). It can be assumed that this metabolite is as stable as the parent when incubated under anaerobic conditions.

Overall summary of laboratory degradation rates in soil

Bentazone

A summary of all estimated persistence (original half-lives) and modelling laboratory half-lives which are normalised to reference conditions of 20 °C temperature and moisture at pF2 is given in Table 7.2/31 for the parent compound bentazone. Only those studies carried out according to a relevant guideline (e.g. OECD or SETAC) were used for the overview. All other studies are considered as supplemental information and were not included in the selection of the laboratory degradation endpoints.

Table 7.2/31 Laboratory half-lives of bentazone at 20 °C and moisture at pF2

Soil	DegT50/DegT90 original [days]	soil type [USDA]	Actual Moisture [%]	Reference moisture (at pF2) [%]	moisture correction factor [-]	DegT50/DegT90 normalized to moisture at pF2 [days]	Source
Bruch West	45.1 / 149.8	sandy loam	9.44	14	0.759	34.2 / 113.7	II A 7.1.1/1 2010/1057318
Bruch West	33.0 / 109.6	sandy loam	11.28	19	0.694	22.9 / 76.1	II A 7.2.1/1 2011/1000621
Li10	43.4 / 144.2	loamy sand	10.28	14	0.806	35.0 / 116.1	
LUFA 2.2	30.9 / 102.6	loamy sand	13.52	14	0.976	30.2 / 100.2	
LUFA 2.3	49.1 / 163.1	sandy loam	10	19	0.638	31.3 / 104.1	
Borstel	16.9 / 56.1	loamy sand	11.6	14	0.877	14.8 / 49.2	Ebert, 2000 2000/1000142
Geometric mean	**30.1 / 114.3**					**20.3 / 89.5**	
Median	**32.0 / 126.9**					**22.9 / 102.1**	
CV	**49%**					**45%**	

The laboratory persistence DegT50 endpoints of bentazone range from 16.9 to 49.1 days with a geometric mean of 30.1 days. The corresponding DegT90 values range from 56.1 to 163.1 days with a geometric mean of 114.3 days.

The laboratory modelling DegT50 endpoints of bentazone range from 14.8 to 35.0 days with a geometric mean of 20.3 days. The corresponding DegT90 values range from 49.2 to 116.1 days with a geometric mean of 89.5 days.

N-methyl-bentazone

A summary of the estimated persistence (original half-lives) and modelling laboratory half-lives which are normalised to reference conditions of 20 °C temperature and moisture at pF2 is given in Table 7.2/32 for N-methyl-bentazone.

Table 7.2/32 Laboratory half-lives of N-methyl-bentazone at 20 °C and moisture at pF2

Soil	DegT50/DegT90 original [days]	soil type [USDA]	Actual Moisture [%]	Reference moisture (at pF2) [%]	moisture correction factor [-]	DegT50/DegT90 normalized to moisture at pF2 [days]	Source
LUFA 2.2	153.0 / 508.3	Loamy sand	19.4	14	1.00	153.0 / 508.3	II A 7.2.3/1 2005/1026922
LUFA 3A	38.0 / 126.2	Loam	20.5	25	0.87	33.0 / 109.8	
PTRL soil	49.0 / 162.8	Clay loam	18.2	28	0.74	36.2 / 120.4	
Geometric mean	**65.8 / 218.6**					**56.8 / 188.7**	
Median	**49.0 / 162.8**					**36.2 / 120.4**	
CV	**79%**					**92%**	

The laboratory persistence DegT50 endpoints of N-methyl-bentazone range from 38.0 to 153.0 days with a geometric mean of 65.8 days. The corresponding DegT90 values range from 126.2 to 508.3 days with a geometric mean of 218.6 days.

The laboratory modelling DegT50 endpoints of N-methyl-bentazone range from 33.0 to 153.0 days with a geometric mean of 56.8 days. The corresponding DegT90 values range from 109.8 to 508.3 days with a geometric mean of 188.7 days.

7.3 Field studies

The calculated field degradation rates of bentazone as listed in the EU review report (2000) were derived from five different trial data sets and were in the range of 4 – 21 d with an arithmetic mean value of 14 days.

Already in 2000, an additional data set from a trial in France (Pouliou) was included in the kinetic analysis and since then used for exposure evaluations. Overall, the dissipation of bentazone in the field was thus investigated in three studies at a total of six trial sites in Germany and France. First-order dissipation DT_{50} values in the field trials ranged from 7 to 22 days.

The estimated DT_{50} values were normalized to the reference temperature of 20°C using a Q_{10} value of 2.2 [Gottesbüren (2001), 2001/1010666]. The normalized field DT_{50} values ranged from 6 to 15 days, with a median value of 9.0 days (Table 7.3/1).

Table 7.3/1 Summary of aerobic degradation rates of bentazone in field soil dissipation studies (Gottesbüren (2001))

Location	Soil Type	pH [-]	Organic carbon [%]	Average air temperature [°C]	DT_{50} (SFO) [d]	Normalized DT_{50} (20°C) [d]
Goch/Nierswalde	sandy loam	5.9	1.8	17.5	14	10
Havixbeck	loamy clay	6.4	1.1	15.6	13	7
Limburgerhof	loamy sand	6.5	1.2	19.0	9	8
Holzen	sandy loam	7.1	1.3	15.2	**22**	15
Stetten	silty clay	6.8	1.3	17.1	17	12
Pouliou	loamy sand	5.7	2.0	18.2	7	6
Median	-				14	**9**

All existing national registrations of bentazone containing products within the EU were based on these values since Annex I inclusion in 2000.

Although it can be assumed that the old bentazone field degradation studies of Schepers and Hesse, 1991 (1991/11611), Hesse and Schepers, 1991 (1991/11761) and Richter, 2000 (2000/1000137) result in reliable half-lives, new studies were initiated due to some deficiencies of the old studies such as only a limited number of parallel samples or the use of old analytical methods in order to satisfy new guidelines.

In 2008, a field dissipation study was carried out at 4 different sites in Europe (see II A 7.3.1/1, Richter and Kuhnke, 2010/1151827), but surface processes were not excluded by the study design. Therefore, according to newer guidance of EFSA (2010), the first sampling points, which were collected before reaching 10 mm of cumulative rainfall, were eliminated from the kinetic evaluation. Due to fast dissipation of bentazone, sufficient data points remained only from two out of four trials for derivation of reliable field $DegT_{50}$ values.

According to the recommendations of the new EFSA guidance on half-lives for modelling purpose (EFSA, 2010), an additional study was performed at four different sites in Europe in 2011 (see II A 7.3.1/2, Richter, 2011/1277036). In this study, surface loss processes such as volatilisation or photolytical degradation were eliminated by irrigation of at least 10 mm immediately after application, leaching the substance from the surface into the bulk soil.

Kinetic evaluation under consideration of recent guidance (FOCUSkinetics, 2006, EFSA 2010) was carried out for all studies (see II A 7.3.1/3, Eickler and Budde, 2011/1277148; II A 7.3.1/4, Matejek and Budde, 2011/1277149; and II A 7.3.1/5, Matejek and Budde, 2011/1277150). A summary of all estimated persistence and modelling half-lives is shown in Table 7.3/28. Since the estimated half-lives from the old studies were in the same range as the new studies they were considered as supportive data and were also taken into account for the derivation of the relevant persistence and modelling endpoints for the exposure assessment.

7.3.1 Soil dissipation testing in a range of representative soils

Report:	II A 7.3.1/1 Richter T., Kuhnke G. 2012(a) Field soil dissipation study of BAS 351 H (Bentazone) in the formulation BAS 351 32 H on bare soil at four different locations in Europe, 2008 BASF DocID 2010/1151827
Guidelines:	SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995); SANCO/825/00 rev. 7 (17 March 2004); SANCO/3029/99 rev. 4 (11 July 2000); EEC 95/36 of 14 July 1995 amending 91/414/EEC; BBA VI 4-1 (December 1986); ECPA Guidance Document on Field Soil Dissipation Studies Aug. 1997
Testing Laboratory and dates:	BASF SE; Limburgerhof; Germany Fed.Rep. 14-Apr-2008 - 19-Nov-2009
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The dissipation of bentazone (BAS 351 H) and its metabolite N-methylbentazone under field conditions was investigated at four sites in Europe representative of Northern and Southern EU conditions. Two trials were performed in Germany, one in Southern France and one in Italy. All sites represent typical regions of agricultural practice.

The product, formulated as an SL, was applied to bare soil in a single application at a nominal rate of 1000 g a.s./ha using a target water volume of 300 L/ha. Applications were conducted between mid-April and end of May 2008 using a calibrated boom sprayer. Dose verification conducted via application monitors yielded an average recovery of 97 % of the target rate over all sites. Directly after application, the plots were not irrigated.

Soil specimens were taken at intervals of up to 115 days and down to a soil depth of 50 cm (except trial site Waldsee, Germany where samples were taken down to 60 cm). Soil cores were cut into sections of 10 cm. Soil segments of the same depth were pooled and homogenized and a representative sub-sample of each depth was taken for residue analysis. All soil specimens were stored at about -18 °C latest 6 hours after sampling and remained frozen until analysis.

Soil specimens were analyzed for bentazone and N-methylbentazone and application monitors for bentazone according to BASF method L0136/01. The analytical method involved methanol/water (50/50, v/v) extraction of the soil. The final determination of the analyte was performed by LC-MS/MS with a limit of quantitation (LOQ) of 0.01 mg/kg. Analysis of soil specimens originating from the treated plots was conducted down to depths where at least two soil segments showed no bentazone (BAS 351 H) and N-methylbentazone residues (< LOQ of 0.01 mg/kg). Soil specimens were analyzed to a maximum of 115 days.

No residues of bentazone and N-methylbentazone above 20 % of the LOQ were detected in any of the untreated control samples, proving that there were no interferences of the untreated soil material with the analytical procedure used. Procedural recovery experiments performed with field soils spiked with bentazone at concentrations of 0.01, 0.1 and 1.0 mg/kg yielded overall mean recovery rates of 87 % for each level, confirming the validity of the analytical method used in this study. Procedural recovery experiments performed with field soils spiked with N-methylbentazone at concentrations of 0.01, 0.1 and 1.0 mg/kg yielded overall mean recovery rates of 98 %, 99 %, and 98 %, respectively, also confirming the validity of the analytical method used in this study.

Bentazone degraded fast in soil under field conditions at all four European sites. At 115 days after treatment, no residues above the LOQ (0.01 mg/kg) could be detected anymore. No residues of N-methylbentazone were found in any soil sample at any site.

Residues of the test substance bentazone were found only in the upper 30 cm of the soil, indicating a moderate tendency of bentazone to reach deeper soil layers.

I. MATERIALS AND METHODS

1. Test material

Test item (formulation):	BAS 351 32 H
Active ingredient (a.i.):	BAS 351 H (Reg.No. 51929)
Type of formulation:	SL
Batch no.:	12654 (certificate of analysis: 346280_10)
Content of active ingredient:	493.3 g/L (nominal 480.0 g/L)
Expiration date:	March 31, 2010

2. Test sites

The dissipation of bentazone and its metabolite N-methylbentazone under field conditions was investigated at four sites in Europe representative of Northern and Southern conditions. Two trial sites were located in Germany (L080157 and L080158), one in France (L080159) and one in Italy (L080160). The site characteristics including the basic soil parameters of the corresponding soil horizons are presented in Table 7.3/2.

Table 7.3/2 Characteristics of the trial sites used to investigate the field dissipation of bentazone

Trial	**L080157**				**L080158**		
Location	**Waldsee, Germany**				**Goch-Nierswalde, Germany**		
Soil properties	0 - 40 cm	40 - 55 cm	55 - 85 cm	85 – 100 cm	0 - 30 cm	30 - 60 cm	60 - 90 cm
Soil texture (DIN 4220)	poor silty sand	poor silty sand	pure sand	poor loamy sand	sandy silt	sandy silt	sandy silt
sand [%]	80.7	77.1	86.4	83.9	17.0	19.9	40.3
silt [%]	16.6	18.4	9.5	10.0	77.4	76.1	57.6
clay [%]	2.7	4.3	4.0	6.0	5.6	4.1	2.1
Soil texture (USDA)	loamy sand	loamy sand	loamy sand	sand	silt loam	silt loam	silt loam
sand [%]	78.7	79.9	86.8	88.9	27.7	26.8	40.1
silt [%]	17.3	16.6	8.3	7.2	66.2	67.3	58.6
clay [%]	4.2	3.5	4.8	3.9	6.0	5.9	1.3
Organic C [%]	0.45	0.20	0.09	0.08	1.34	0.27	0.23
Organic matter [%] *	0.78	0.34	0.16	0.14	2.31	0.47	0.40
pH [H_2O]	5.5	6.1	6.1	6.1	6.9	6.7	5.2
pH [$CaCl_2$]	4.6	4.9	5.2	5.2	6.3	6.3	4.6
CEC [mval Ba/100g dry weight]	6.9	1.6	2.3	2.2	5.5	1.7	7.3
MWHC [g/100g dry weight]	30.1	25.9	24.6	24.9	35.0	31.2	33.1

Trial	**L080159**			**L080160**		
Location	**Meauzac, Southern France**			**Poggio Renatico, Italy**		
Soil properties	0 - 20 cm	20 - 50 cm	50 - 90 cm	0 - 30 cm	30 - 50 cm	50 - 90 cm
Soil texture (DIN 4220)	silty-loamy sand	silty-loamy sand	sandy loamy silt	medium clay silt	high clay silt	medium clay silt
sand [%]	49.0	43.0	24.1	4.9	1.0	15.9
silt [%]	42.9	43.2	61.0	78.7	78.0	71.0
clay [%]	8.1	13.8	14.9	16.4	21.1	13.1
Soil texture (USDA)	loam	loam	loam	silt loam	silt loam	silt loam
sand [%]	50.5	45.4	28.3	8.3	1.9	21.3
silt [%]	41.5	44.4	49.6	76.5	76.8	66.0
clay [%]	8.2	10.3	22.2	15.2	21.3	12.7
Organic C [%]	2.91	0.58	0.32	1.63	0.96	0.52
Organic matter [%] *	5.02	1.00	0.55	2.81	1.66	0.90
pH [H_2O]	4.7	5.2	5.3	8.3	8.0	8.2
pH [$CaCl_2$]	4.4	4.3	4.5	7.7	7.8	7.8
CEC [mval Ba/100g dry weight]	8.9	6.7	13.7	20.8	27.9	13.3
MWHC [g/100g dry weight]	38.8	36.0	41.4	46.0	48.9	43.0

* organic matter = organic carbon x 1.724

CEC = cation exchange capacity; MWHC = maximum water holding capacity

The selected trial sites represented typical regions of agricultural practice and had been under cultivation for many years. The sites were flat without any significant slope. Before commencement of the first sampling, the soil at each trial site was prepared as for sowing and was rolled if considered necessary, but then was left fallow.

No product containing the test item had been used on the test plots in the last three years, nor any soil disinfectants (e.g. Dazomet or Metam) within the previous year. The management history of the trial sites is provided in Table 7.3/3.

Table 7.3/3 Management history of the trial sites in the previous years

Trial	Location	Year	Crops grown	Pesticides used
L080157	Waldsee, Germany	2007	carrots	Pendimethalin
		2007	carrots	Dimethoat
		2006	closing	No pesticides applied
		2005	closing	No pesticides applied
L080158	Goch-Nierswalde, Germany	2007	Bare soil	Glyphosate
		2006	Bare soil	Pentiopyrad
		2005	Sunflower	No pesticides applied
L080159	Meauzac, Southern France	2007	Non cropped	No pesticides applied
		2006	Non cropped	No pesticides applied
		2005	Apples	No pesticides applied
L080160	Poggio Renatico, Italy	2007	Sorghum	Terbutilazine, Propachlor
		2006	Soybean	Imazamox, Tifensulfuron-methyl
		2005	Winter wheat	Clodinafop-propargyl, Cloquitocet-mexyl, Fluroxypir, Pirimicarb, Tebuconazole

3. Experimental treatments

The product, formulated as an SL, was broadcast applied to bare soil in a single application at a nominal rate of 1000 g a.s./ha using a target water volume of 300 L/ha. The trial L080159 in Meauzac was applied with only 250 L/ha. The applications were conducted between mid-April to end of May 2008 using a calibrated boom sprayer. Treated plots were four-fold replicated with plot size ranging from 256 to 320 m^2. The actual application rates determined by quantifying the amount of spray discharged ranged from 947.77 to 1053.5 g/ ha for the trials in Waldsee, Goch and Poggio Renatico, and between 796.5 to 859.7 g a.s./ha in Meauzac. The reduced rate in Meauzac was a result of a mistake during application speed calculation and the reduced water volume. In addition, the dose was verified by means of sampling Petri dishes filled with standard soil LUFA 2.2 (approximately 50 g per dish, sieved to 2 mm) placed at the time of application at the treated plots. Details of application are presented in Table 7.3/4.

Table 7.3/4 Application parameters of field trial sites treated with bentazone

Trial Country	Test item/ Nominal content/ Formulation type	Application Method	No. of appli-cations	Application rate per treatment			No. of treated replicates	Application date
				nominal [g a.s./ha]	actual* [g a.s./ha]	dose verifi-cation**		
L080157 Waldsee Germany	BAS 351 32 H 1000 g a.s./L SL	broadcast spray to bare soil	1	1000	990	100% of nominal rate	4 (64 m^2 each)	27-May -2008
L080158 Goch-Nierswalde Germany	BAS 351 32 H 1000 g a.s./L SL	broadcast spray to bare soil	1	1000	983	100% of nominal rate	4 (80 m^2 each)	15-April-2008
L080159 Meauzac Southern France	BAS 351 32 H 1000 g a.s./L SL	broadcast spray to bare soil	1	1000	822	89% of nominal rate	4 (78 m^2 each)	20-May-2008
L080160 Poggio Renatico Italy	BAS 351 32 H 1000 g a.s./L SL	broadcast spray to bare soil	1	1000	1039	98% of nominal rate	4 (66 m^2 each)	06-May-2008

* determined by calculation of spray liquid applied; mean of four replicates

** determined by means of Petri dishes filled with soil, corrected for procedural recovery

The test plots in Germany were not irrigated. The test plots in Meauzac, France and Poggio Renatico, Italy were irrigated matching the amount evaporated (non-GLP). The irrigation was performed with sprinklers at six and nine occations distributed evenly over the study period at rates from 3.8 to 12 mm not earlier than two weeks after application. Irrigation was verified by placing three pluviometers in each replicate of the treated plot and two pluviometers in the control plot and calculating the average of the catches.

No tillage or fertilization was performed during the course of the study from first to last sampling and no crops were grown throughout any of the trials. The plots were kept free of vegetation via the application of glyphosate.

Climatic conditions were based on records of appropriate weather stations located on-site or at a distance of maximal 12 km from site. Monthly summary results on temperature, precipitation and irrigation are presented in Table 7.3/5.

Table 7.3/5 Summary of climatic conditions at the trial sites used to investigate the field dissipation of bentazone

Trial	L080157		L080158		L080159		L080160	
Location	Waldsee		Goch-Nierswalde		Meauzac		Poggio Renatico	
	Germany		Germany		Southern France		Italy	
Climatic conditions	T_{mean} Air [°C]	Prec. [mm]	T_{mean} Air [°C]	Prec. [mm]	T_{mean} Air [°C]	Prec. [mm]	T_{mean} Air [°C]	Prec. [mm]
Month								
Apr 08	n.a.	n.a.	10.2	27.2	n.a.	n.a.	n.a.	n.a.
May 08	22.8	9.9	15.3	53.0	16.5	80.3	17.9	100.2
Jun 08	19.7	33.3	16.2	60.8	18.9	53.2	22.0	171.7
Jul 08	20.8	40.5	17.5	114.4	20.5	87.3	24.6	60.0
Aug 08	19.7	86.2	18.9	29.0	20.2	36.7	24.5	29.2
Sep 08	15.9	42.4	n.a.	n.a.	19.2	31.3	n.a.	n.a.

4. Sampling

Replicate soil specimens (20 per treated plot and 20 per control plot) were taken at intervals up to about 115 days and down to a soil depth of up to 50 cm (Waldsee, Germany 60 cm). At day 0, immediately after application, the treated plots were sampled down to 10 cm only (except Waldsee – here samples were taken down to 60 cm). The detailed sampling intervals are presented in Table 7.3/6.

Table 7.3/6 Summary of sampling intervals at each field trial site

Trial	Country	Sampling intervals [days after treatment]
L080157	Germany	-1, 0, 1, 3, 6, 14, 27, 65, 113
L080158	Germany	-1, 0, 1, 3, 7, 13, 31, 58, 115
L080159	France (South)	-1, 0, 1, 3, 8, 14, 31, 56, 115
L080160	Italy	-3, 0, 1, 3, 7, 16, 34, 63, 115

Untreated specimens were collected from the control plot on two occasions, one day before the application (Poggio Renatico, Italy, 3 days before application) and on the final day of sampling. The 20 specimens were taken randomly from half of the untreated plot each time and pooled according to soil depth. Specimens were taken using a common soil probe equipped with a plastic liner of 2.5 to 5 cm diameter at the sampling occasion before application. At the final day of sampling, the 0-10 cm layer was collected separately using a metal tube of greater diameter as is described below for the treated plots.

Treated soil specimens were taken randomly from five points of each of the four treated subplots A to D and pooled together according to depth. All soil specimens from 0-10 cm depth collected from the treated plots were taken separately using a stainless steel tube of minimum 7.2 and maximum 9.8 cm diameter which left a hole by pressing a metal tube into the ground and collecting the soil with a spoon or similar device. Soil specimens deeper than 10 cm were collected through the center of the excavation hole contained by the guard collar, using a common soil probe fitted with a plastic liner of diameter 2.5 to 5 cm. Sampling of these cores was conducted in one run or in up to two consecutive steps.

All soil specimens were stored at or below -18°C within less than 6 hours of being taken and remained frozen until analysis.

5. Description of analytical procedure

The soil specimen were analyzed for bentazone and its metabolite N-methylbentazone and Petri dish specimens for bentazone using BASF method L0136/01. A 5 g soil sample was extracted with methanol/water (50/50, v/v). A 5 mL aliquot of the extract was centrifuged and the extract taken directly or diluted with methanol/water (50/50, v/v) to the appropriate final volume and measured by Ultra Performance Liquid Chromatography (UPLC)-MS/MS. The limit of quantification (LOQ) was 0.01 mg/kg for each of the analytes. In case of the Petri dish specimens, the amount of soil (50 g) was extracted with an extended amount of methanol/water (50/50, v/v).

Analysis of field soil specimens originating from the treated plots was conducted down to a soil depth ensuring that at least two soil segments were free of bentazone and N-methylbentazone (< LOQ of 0.01 mg/kg). Beyond that, no samples were analyzed. Residue analyses were performed until the last soil sampling at 115 days after treatment (DAT).

6. Storage stability experiments

Storage stability of bentazone in soil at a temperature of <-5 °C was tested as part of an earlier study [Stewart J.: Freezer Storage Stability of Bentazon in Soil; November 1994; study code: A8804; BASF DocID: 1994/5163]. In August 2009, a two-year storage stability study in soil for bentazone and N-methylbentazone in soil was started for 24 months at a temperature of about -20 °C [Tornisielo A., Sacchi R.: Investigation of the Storage Stability of BAS 351 H (Bentazon) and its Metabolite BH 351-N-Me in soil samples at about -20 °C; study code: 376443; study is still ongoing].

II. RESULTS AND DISCUSSION

A. APPLICATION VERIFICATION

Procedural recovery experiments were conducted with standard soil LUFA 2.2. Fortification levels of bentazone were at 0.01 and 18 mg/kg. The fortification experiments yielded recoveries of 106 ± 11.3 % (mean ± RSD, n = 4) for the fortification level 0.01 mg/kg and 107 ± 3.1 % (mean ± RSD, n = 8) for the fortification level 18 mg/kg. Residues in blank samples were not detectable above 20% of the LOQ.

Residue levels of bentazone achieved on extraction and analysis of the application monitors (Petri dishes filled with soil LUFA 2.2) were corrected for procedural recoveries but converted into residue rates (in g/ha) taking into account the area of the Petri dishes (91.6 cm²).

As a result, the obtained rates for the individual trials ranged from 894 ± 196 to 1001 ± 188 g/ha representing 89-100% of the target application rate (see also Table 7.3/4).

B. FINDINGS

Untreated soil specimens (control samples) of the respective soil depths from each trial were analyzed for residues of bentazone and its metabolite N-methylbentazone. In all cases the concentration of bentazone and its metabolite N-methylbentazone in the unfortified control samples was below 20 % of the LOQ indicating the absence of interferences.

Furthermore, untreated soil was fortified with bentazone and N-methylbentazone at concentrations of 0.01, 0.1, and 1.0 mg/kg. The fortification experiments yielded average recoveries for bentazone of 87 ± 6.4 %, 87 ± 5.7 % and 87 ± 7.4 % for fortifications of 0.01, 0.1, and 1.0 mg/kg, respectively. Recovery values for N-methylbentazone (mean ± RSD) were 98 ± 5.8 %, 99 ± 4.9 %, 98 ± 4.9 % for fortifications of 0.01, 0.1, and 1.0 mg/kg, respectively. Average results are summarized in Table 7.3/7.

Table 7.3/7 Method procedural recoveries

Analyte	Fortification levels [mg/kg]	Average recovery [%] ± Standard deviation*	n
Bentazone	0.01, 0.1, 1.0	86.8 ± 5.4	38
N-methylbentazone	0.01, 0.1, 1.0	98.2 ± 5.3	38

* mean values are across all fortification levels and all soils

These data prove that the analytical method applied is suitable to accurately determine residues of the two analytes in soil down to a concentration of 0.01 mg/kg of each analyte.

Residue levels of bentazone in mg/kg dry soil were converted to residue rates in g/ha, taking into account the actual dry soil density of the field sample, and were summed up for all depths between 0 and 50 cm analyzed. All residue values presented in the table below (Table 7.3/8) are related to the dry weight of the soil and were not corrected for procedural recoveries.

Table 7.3/8 Total residues of bentazone under field conditions in soil calculated to g/ha based on actual dry soil density and summed up for all depths analyzed

Trial Country	L080157 Waldsee, Germany	L080158 Goch-Nierswalde, Germany	L080159 Meauzac, France	L080160 Poggio Renatico, Italy
days after treatment	[g/ha]	[g/ha]	[g/ha]	[g/ha]
0	537	684	560	890
1	624	701	326	592
3	466	564	383	480
6-8	329	287	97	411
13-16	200	108	12	162
27-34	70	26	0	53
56-65	14	0	0	10
113-115	0	0	0	0

Bentazone degraded at all four European field sites. By end of the study after about 115 days, no residues above the LOQ (0.01 mg/kg) were detectable.

The main proportion of bentazone residues was always measured in the top 0-10 cm soil layer. Only small amounts of bentazone above 0.01 mg/kg were detected occasionally in the 10-20 cm layer and only once also in the 20-30 cm layer (at the trial site Poggio Renatico). No residues above the LOQ were detected below 30 cm in any sample. Altogether, it can be concluded that bentazone show a moderate tendency to move into deeper soil layers.

The metabolite N-methylbentazone could not be detected in any soil sample.

The comprehensive analytical data (in mg/kg dry soil) of bentazone and N-methylbentazone residues in the individual soil layers are presented in detail in the summary tables of Document L II, 7.3.

A detailed kinetic evaluation of the field trials is presented in a separate modeling report (see II A, 7.3.1/3).

Storage stability

In storage stability experiments an average recovery of 81 % of the initial bentazone was obtained after 24 months of storage in frozen soil (<-5°C) indicating that the compound is stable during the period investigated.

Preliminary experiments from the ongoing second stability study indicate that both compounds are stable in frozen soil for at least 2 years.

III. Conclusions

Bentazone degraded in soil under field conditions at all four European sites down to a level with no residues above the LOQ (0.01 mg/kg) at about 115 days at the latest. The calculations are the subject of a separate modeling report. The main proportion of bentazone residues was always measured in the top 0-10 cm soil layer. Only small amounts of bentazone above 0.01 mg/kg were found occasionally in the 10-20 cm layer and only once also in the 20-30 cm layer (at the trial site Poggio Renatico). No residues above the LOQ were detected below 30 cm in any sample, indicating a moderate tendency of bentazone to reach deeper soil layers. N-methylbentazone was not detected in any of the soil samples.

Report:	II A 7.3.1/2 Richter T. 2012(a) Field soil dissipation study of BAS 351 H (Bentazone) in the formulation BAS 351 32 H on bare soil at four different sites in Europe, 2011 BASF DocID 2011/1277036
Guidelines:	EPA 835.6100; SETAC Procedures for assessing the environmental fate and ecotoxicity for pesticides (March 1995); EFSA Guidance to obtain DT50 values in soil (2010); SANCO/825/00 rev. 7 (17 March 2004); SANCO/3029/99 rev. 4 (11 July 2000); NAFTA Guidance Document for Conducting Terrestrial Field Dissipation Studies Regulatory Directive DIR2006-01 (March 2006)
Testing Laboratory and dates:	BASF SE; Limburgerhof; Germany Fed.Rep. 30-Mar-2011 - 18-Nov-2011
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The dissipation of bentazone (BAS 351 H) and its metabolite N-methylbentazone under field conditions was investigated at four sites in Europe representative of Northern and Southern EU conditions. One trial was performed in Germany, one each in Northern and Southern France and one in Spain. All sites represent typical regions of agricultural practice.

The product BAS 351 32 H (Basagran), formulated as an SL, was broadcast applied to bare soil in a single application at a nominal rate of 1000 g a.s./ha using a target water volume of 300 L/ha. Applications were conducted between beginning of April and end of May 2011 using a calibrated boom sprayer. The actual application rates determined by quantifying the amount of spray discharged ranged from 1002 to 1074 g a.s./ha for all trials with an average of 1043 g a.s./ha. In addition, aliquots of the application solutions were taken for analysis before and after application. They yielded 89 to 121 % of the intended concentration. Dose verification conducted via Petri dishes filled with soil yielded an average recovery of 1048 g a.s./ha equivalent to 105 % of the target rate over all sites.

Immediately after application, the plots were irrigated in order to exclude residues from surface degradation processes like photolysis or volatilization. Additional irrigation was conducted only for three of the four trials.

No tillage or fertilization was performed during the course of the study and no crops were grown throughout any of the trials. The plots were kept free of vegetation via the application of glyphosate.

Soil specimens were taken at intervals of up to nominal 120 days and down to a soil depth of 50 cm. Soil cores were cut into sections of 10 cm. Soil segments of the same sampling depth and replicate were pooled and homogenized and a representative sub-sample of each depth was taken for residue analysis. All soil specimens were stored at about -18 °C within a maximum of 9 hours and 30 minutes of being taken and remained frozen until analysis.

In order to prove the stability of residues during storage and shipment, shipment verification samples were prepared at samplings 0 and 30 DAT by fortifying untreated soil from the field site with a known amount of bentazone. These samples were stored and shipped together with residue samples taken from the field at the same time until analysis. As a result, percentages from 92 % to 107 % of the prepared concentration were measured confirming residue stability during all storage and shipment procedures.

Soil specimens were analyzed for bentazone and N-methylbentazone and application monitors for bentazone according to BASF method L0136/01. The analytical method involved methanol/water (50/50, v/v) extraction of the soil. The final determination of the analyte was performed by LC-MS/MS with a limit of quantitation (LOQ) of 0.01 mg/kg. Analysis of soil specimens originating from the treated plots was conducted down to a depth ensuring that at least two soil segments were free of bentazone and N-methylbentazone (< LOQ of 0.01 mg/kg). Soil specimens were analyzed for a maximum period of 127 days after application.

No residues of bentazone or N-methylbentazone above the LOQ were detected in any of the untreated control samples, proving that there were no interferences of the untreated soil material with the analytical procedure used. Procedural recovery experiments performed with field soils spiked with bentazone at concentrations of 0.01, 0.1, 1.0, 2.0 and 12.0 mg/kg yielded mean recovery rates per fortification level in the range of 83 % to 101 % with relative standard deviations below 9.1 %, confirming the validity of the analytical method used in this study. Procedural recovery experiments performed with field soils spiked with N-methylbentazone at equal concentration levels yielded mean recovery rates per level from 93 % to 105 % with relative standard deviations below 9.1 %, also confirming the validity of the analytical method used in this study.

Residue levels of bentazone in mg/kg dry soil were converted to residue rates in g/ha taking into account the actual dry soil density of the field sample and were summed up for all depths between 0 and 50 cm analyzed. Residue values were not corrected for procedural recoveries.

Bentazone degraded in soil under field conditions at all four European sites with no residues above the LOQ (0.01 mg/kg) left after about 120 days. Very low concentrations of N-methylbentazone were found in the 0-10 cm soil layer of only one trial. Residues of bentazone were found predominantly in the upper 20 cm of the soil, and never at a depth greater than 40 cm.

In a still ongoing study, the stability of residues of bentazone and N-methylbentazone in soil after 24 months of freezer storage (at about -20 °C) was investigated. Both compounds were found to be stable for the whole period tested. This period covers the maximum storage time from sampling to analysis of samples collected during the course of the present field soil dissipation study.

I. MATERIALS AND METHODS

1. Test material

Test item (formulation):	BAS 351 32 H
Active ingredient (a.i.):	BAS 351 H (Reg.No. 51929)
Type of formulation:	SL
Batch no.:	70896356PO
Content of active ingredient:	485.8 g/L (nominal 480.0 g/L)
Expiration date:	March 31, 2013

2. Test sites

The dissipation of bentazone and its metabolite N-methylbentazone under field conditions was investigated at four sites in Europe representative of northern and southern EU conditions. One trial site was located in Germany (L110237), two in France (L110238 and L110240) and one in Spain (L110239). Soil characteristics are presented in Table 7.3/9.

Table 7.3/9 Characteristics of the trial sites used to investigate the field dissipation of Bentazone and its metabolite N-methylbentazone

Trial	**L110237**			**L110238**	
Location	**Goch-Nierswalde, Germany**			**Stotzheim, Northern France**	
Soil properties	0 - 30 cm	30 - 60 cm	60 - 90 cm	0 - 45 cm	45 - 90 cm
Soil class (DIN 4220)	Clay silt	Clay silt	Sandy silt	Clay silt	Clay silt
clay [%]	9.7	8.9	7.6	21.2	9.7
silt [%]	74.9	76.2	52.6	71.4	73.2
sand [%]	15.4	14.9	39.9	7.5	17.1
Soil class (USDA)	Silt loam	Silt loam	Silt loam	Silt loam	Silt loam
clay [%]	9.7	8.9	7.6	21.2	9.7
silt [%]	73.1	72.9	50.9	71.4	72.2
sand [%]	17.2	18.2	41.5	7.5	18.1
Total organic C [%]	1.89	0.44	0.21	0.97	0.29
Organic matter [%] *	3.26	0.76	0.36	1.67	0.50
pH [$CaCl_2$]	5.9	6.0	5.3	7.4	7.7
pH [H_2O]	6.3	6.8	6.3	8.1	8.6
CEC [$cmol^+$ /kg]	6.6	2.7	2.2	13.8	8.9
MWHC [g/100g dry weight]	36.7	29.7	23.5	34.1	32.3
Dry bulk density [g/cm^3]	1.244	-	-	1.164	-
pF 2.0** [$g_{\text{soil moisture}}$ / $g_{\text{dry soil}}$]	0.263 (26.3%)	0.256 (25.6%)	0.204 (20.4%)	0.270 (27%)	0.283 (28.3%)
pF 2.5** [$g_{\text{soil moisture}}$ / $g_{\text{dry soil}}$]	0.232 (23.2%)	0.147 (14.7%)	0.113 (11.3%)	0.194 (19.4%)	0.189 (18.9%)
Trial	**L110239**			**L110240**	
Location	**Utrera, Spain**			**Barry d´Islemade, SouthernFrance**	
Soil properties	0 - 15 cm	15 - 30 cm	30 - 90 cm	0 - 40 cm	40 - 90 cm
Soil class (DIN 4220)	Clay sand	Clay sand	Sandy clay	Loamy sand	Sandy loam
clay [%]	5.4	5.3	37.5	10.8	21.7
silt [%]	6.8	7.3	5.2	24.2	19.4
sand [%]	87.7	87.4	57.2	65.1	58.9
Soil class (USDA)	Sand	Sand	Sandy clay	Sandy loam	Sandy clay loam
clay [%]	5.4	5.3	37.5	10.8	21.7
silt [%]	4.2	3.6	4.4	22.0	17.9
sand [%]	90.3	91.1	58.0	67.2	60.4
Total organic C [%]	0.37	0.25	0.34	0.55	0.26
Organic matter [%] *	0.64	0.43	0.59	0.95	0.45
pH [$CaCl_2$]	6.7	6.7	5.9	5.8	6.4
pH [H_2O]	7.6	7.7	6.7	6.4	6.9
CEC [$cmol^+$ /kg]	5.6	6.0	24.9	5.2	8.7
MWHC [g/100g dry weight]	23.6	24.8	39.6	25.3	28.9
Dry bulk density [g/cm^3]	1.660	-	-	1.276	
pF 2.0** [$g_{\text{soil moisture}}$ / $g_{\text{dry soil}}$]	0.149 (14.9%)	0.143 (14.3%)	0.343 (34.3%)	0.183 (18.3%)	0.222 (22.2%)
pF 2.5** [$g_{\text{soil moisture}}$ / $g_{\text{dry soil}}$]	0.106 (10.6%)	0.113 (11.3%9	0.278 (27,8%)	0.138 (13.8%)	0.162 (16.2%)

* organic matter = organic carbon x 1.724

CEC = effective cation exchange capacity

MWHC = maximum water holding capacity

** Water retention characteristics, calculated on base of mass

The selected fields represented typical regions of agricultural practice, especially for growing peas and had been under cultivation for many years. The sites were flat without any significant slope. Before commencement of the first sampling, the soil at each trial site was prepared as for sowing and was rolled if considered necessary, but then was left fallow.

No product containing the test item a.s. had been used on the test plots in the last three years, nor were any soil disinfectants (e.g. Dazomet or Metam) applied within the previous year. The crop and pesticide history of the trial sites is presented in Table 7.3/10.

Table 7.3/10 Management history of the trial sites in the previous years

Trial	Location	Year	Crops grown	Pesticides used
L110237	Goch-Nierswalde, Germany	2008	No data available	No data available
		2009	No data available	No data available
		2010	Alfalfa	Lentagran (2.0 kg/ha); a.i. Pyridat 450 g/kg
L110238	Stotzheim, France (North)	2008	Winter wheat	Epiconazole
		2009	Vine nursery	Oxyfluorfen, Propyzamide, Oryzalin, Diuron, Glufosinate-ammonium, Fosetyl-ammonium, Folpet, Cymoxanil, Chlorpyriphos-methyl, Dimethomorph, Chlorpyriphos-methyl, Fosetyl-aluminium, Mandipropamid, Sulphur micro, Copper oxychloide
		2010	Maize	Nicosulfuron, mesotrione, dicamba
		2011 *	none	Glyphosate
L110239	Utrera, Spain	2008	Non cropped	No pesticides applied
		2009	Phacelia tanacetifolia	No pesticides applied
		2010	Wheat	Pendimethalin
L110240	Barry d´Islemade, France (South)	2008	Winter oilseed rape	Napropamide, Clomazone, Dimethachlor
		2009	Winter wheat	Pyroclastrobin, Epoxiconazole, Kresoxim-methyl
		2010	Field beans	Pendimethalin, Aclonifen
		2011 *	none	none

*until start of study

3. Experimental treatments

The trial area at each site was divided into two plots, one untreated control plot (size: 30-45 m^2) and one treated plot (size: 198-288 m^2) which consisted of three equal sized subplots A, B and C that were assigned for replicates.

The product, formulated as an SL, was broadcast applied to bare soil in a single application at a nominal rate of 1000 g a.s./ha using a target water volume of 300 L/ha. The applications were conducted between beginning of April and end of May 2011 using a calibrated boom sprayer. Treated plots were three-fold replicated with plot size ranging from 198 to 288 m^2. For each replicate, a separate spray mixture was prepared and the test item was applied to each subplot individually. About 10 mL aliquots of the application solutions were taken before and after application of each individual subplot. The actual application rates were determined by quantifying the amount of spray discharged and are given in Table 7.3/11. In addition, the dose was verified by means of sampling Petri dishes filled with Li 10 soil (approximately 50 g per dish, sieved to 2 mm). The Petri dishes with an inner diameter of 10.8 cm were placed on the treated plot (ten in each subplot) before application. On completion of the application, the Petri dishes were sealed with a lid, taped up and frozen until analysis. The results of analysis and further details of application are given in Table 7.3/11.

Table 7.3/11 Application parameters of field trial sites treated with BAS 351 32 H (SL)

Trial Country	No. of appli-cations	Application Method	Application rate per treatment				No. of treated replicates	Application date
			nominal [g a.s./ha]	Subplot	actual* [g a.s./ha]	dose verifi-cation**		
L110237 Goch-Nierswalde Germany	1	broadcast spray to bare soil	1000	A B C	1074 1011 1062	1080 976 933	3 (81 m^2 each)	26-April -2011
LL110238 Stotzheim France (N)	1	broadcast spray to bare soil	1000	A B C	1060 1038 1070	1103 1052 1125	3 (90 m^2 each)	23-May-2011
L110239 Utrera Spain	1	broadcast spray to bare soil	1000	A B C	1035 1025 1061	1007 975 1077	3 (66 m^2 each)	01-April-2011
L110240 Barry d' Islemade France (S)	1	broadcast spray to bare soil	1000	A B C	1069 1009 1002	1068 1112 1067	3 (96 m^2 each)	10-May -2011

* determined by calculation of spray liquid applied
** determined by means of Petri dishes filled with soil [g a.s./ha]

No tillage or fertilization was performed during the course of the study from first to last sampling and no crops were grown throughout any of the trials. The plots were kept free of vegetation via the application of glyphosate.

All plots were irrigated at least once. Irrigation took place at least after application of test item and sampling of Petri dishes but before first field soil sampling. Amounts applied on the treated plot during the course of the study for Goch-Nierswalde were 123 mm, for Stotzheim 17.5 mm, for Utrera 190.8 mm and for Barry d´Islemade 109 mm. The test plots in Goch-Nierswalde (Germany), Stotzheim (Northern France) and Utrera (Spain) were irrigated matching the amount of precipitation occurring under normal conditions. Therefore, the actual precipitation at the trial site was checked three times per month (about every 10 days) and compared to the historical rainfall in the region (historical data based on a period of at least 10 years). If actual incremental values for each 10-day period were lower than 110% of historical values, the missing amount of water was applied to the field.

For Barry d´Islemade (Southern France) the irrigated amount depended on the amount evaporated at the bare soil plot. Therefore, the actual precipitation at the trial site was checked three times per month (about every 10 days) and compared to the actual evaporation at the plots which was derived from daily reference evapotranspiration (ETo). The irrigation was performed manually or with sprinklers.

Irrigation was verified by placing five pluviometers (at Barry d´Islemade only one) in each replicate of the treated plot. The control plot was irrigated according to the treated plot and mean rates were calculated.

Climatic conditions were based on records of appropriate weather stations located on-site. A summary of monthly results on temperature and precipitation is presented in Table 7.3/12.

Table 7.3/12 Summary of climatic conditions at the trial sites used to investigate the field dissipation of bentazone and its metabolite N-methylbentazone

Trial	**L110237**		**L110238**		**L110239**		**L110240**	
Location	**Goch-Nierswalde**		**Stotzheim**		**Utrera**		**Barry d´Islemade**	
	Germany		**Northern France**		**Spain**		**Southern France**	
Climatic conditions	T_{mean} Air [°C]	Prec. [mm]	T_{mean} Air [°C]	Prec. [mm]	T_{mean} Air [°C]	Prec. [mm]	T_{mean} Air [°C]	Prec. [mm]
Month*		∑		∑		∑		∑
Apr 2011	13.6	15.4	-	-	19.8	77.4	-	-
May 2011	14.2	28.0	16.5	7.8	23.4	23.8	18.1	22.2
Jun 2011	16.1	89.0	17.3	102.0	26.9	2.4	18.7	41.6
Jul 2011	15.6	112.4	16.3	97.8	27.6	0.0	19.5	146.2
Aug 2011	16.9	136.2	18.5	95.2	-	-	22.1	28.8
Sep 2011	-	-	17.7	68.0	-	-	21.5	12.6

*Weather data refer to time period from start of trial (day of application) until end of trial (day of last sampling)

4. Sampling

Replicate soil specimens (8 per treated subplot and 10 to 15 per control plot) were taken at intervals up to 127 days and down to a soil depth of 50 cm. At day 0, immediately after application, the treated plots were sampled down to 10 cm only. The detailed sampling intervals are presented in Table 7.3/13.

Table 7.3/13 Summary of sampling intervals at each field trial site

Trial	Country	Sampling intervals [days after treatment]
L110237	Germany	-3, 0, 1, 3, 7, 14, 30, 55, 107, 127
L110238	France (North)	-10, 0, 1, 3, 8, 16, 30, 57, 91, 115
L110239	Spain	-1, 0, 1, 3, 6, 14, 27, 61, 116
L110240	France (South)	0*, 0, 1, 3, 7, 14, 30, 56, 94, 127

* before application

Untreated specimens were collected from the control plot at two occasions, the first time between zero and ten days before application and a second time on the final day of sampling. The 15 cores for each sampling were taken randomly from a defined part of the untreated plot and pooled according to soil depth. Specimens were taken using a common soil probe (Humax corer) equipped with a plastic liner of 4.4 to 5 cm diameter or a metal tube of 7.2 to 8.3 cm diameter or a combination of both as is described below for the treated plots.

Treated soil specimens were taken randomly from eight points of each of the three treated subplots A to C and pooled according to subplot and depth. All soil specimens from 0-10 cm depth collected from the treated plots were taken separately using a metal tube of minimum 7.2 and maximum 8.3 cm diameter which was pressed into the ground and collecting the soil with a spoon or similar device. Soil specimens deeper than 10 cm were collected through the center of the excavation hole contained by the metal tube, using a common soil probe (Humax corer) fitted with a plastic liner of diameter 4.4 to 5 cm. Sampling of these cores was conducted in one run or in up to two consecutive steps.

Immediately after sampling and before freezing, all soil cores were sectioned into segments of 10 cm and pooled by depth. In addition to the main sampling, a second complete sampling (double sampling) was generally carried out. These reserve samples were not sectioned but directly put into the freezers at the field test sites.

All treated soil specimens (including the Petri dish samples, main samples and double samples) were placed into freezer storage at about -18 °C within a maximum of 9 hours, 30 minutes of being taken. The specimens remained frozen until analysis.

5. Description of analytical procedure

Field soil and shipment verification samples were analyzed for bentazone and N-methylbentazone and Petri dish samples for bentazone using BASF method L0136/01. A 5 g soil sample was extracted with 50 mL methanol/water (50/50, v/v). A 5 mL aliquot of the extract was centrifuged and the extract taken directly or diluted with methanol/water (50/50, v/v) to the appropriate final volume and measured by Liquid Chromatography (LC)-MS/MS. In case of the Petri dish and shipping verification specimens, the amount of soil (50 g and 20 g, respectively) was extracted with an extended amount of methanol/water (50/50, v/v). Additionally, bottom and lid of each dish were rinsed with solvent. The limit of quantification (LOQ) was 0.01 mg/kg for each of the two analytes.

Analysis of soil specimens originating from the treated plots was conducted down to a depth ensuring that at least two soil segments were free of bentazone and N-methylbentazone (< LOQ of 0.01 mg/kg). Beyond that, no samples were analyzed. Residue analyses were performed until at least one residue free sampling (residues of all analyzed horizons < LOQ) was achieved.

6. Storage stability experiments

Storage stability of bentazone in soil at a temperature of <-5 °C was tested as part of an earlier study [Stewart J.: Freezer Storage Stability of Bentazon in Soil; November 1994; study code: A8804; BASF DocID: 1994/5163]. In August 2009, a two-year storage stability study in soil for bentazone and N-methylbentazone in soil was started for 24 months at a temperature of about -20 °C [Tornisielo A., Sacchi R.: Investigation of the Storage Stability of BAS 351 H (Bentazon) and its Metabolite BH 351-N-Me in soil samples at about -20 °C; study code: 376443; study is still ongoing].

7. Shipment verification samples

At 0 and 30 DAT, shipment verification samples were prepared at all field sites. Four samples with 20 g of untreated soil from the field site were weighed into amber glass bottles. Three of them were spiked with 1 mL of a solution containing bentazone (as pure substance) at a concentration of 3 µg/mL in methanol + water (50 + 50, v/v) resulting in fortification samples of 0.15 mg/kg. The fourth bottle with soil remained untreated as control sample. These samples were generally stored and shipped along with the field samples, which were taken at the same day.

8. Calculation of dissipation times

A detailed kinetic evaluation of the degradation behavior of bentazone in the four European field soils is presented in a separate modeling report (see M II, 7.3.1/4).

II. RESULTS AND DISCUSSION

A. APPLICATION VERIFICATION

Petri dishes filled with 50 g soil were used as application monitors. Residue levels of bentazone achieved on extraction and analysis of the application monitors were corrected for procedural recovery (see below) and then converted into application rates (in g/ha) taking into account the area of the Petri dishes (91.6 cm²). The mean application rates (corrected for procedural recovery, n=10) are given in Table 7.3/11.

Within each analytical series of the application monitor analysis, procedural recovery experiments were performed to prove the reliability of the analytical method. The recovery experiments were carried out with untreated soil, either with the same type of soil as was used in the Petri dishes placed in the field or with untreated soil from trial L110237. The fortification levels were about 18 mg/kg BAS 351 H. The fortification experiments had a recovery of 97.6 ± 7.6 % (mean ± RSD, n = 22) for that fortification level. Residues in blank samples were not detectable above the LOQ.

B. FINDINGS

Untreated soil specimens (control samples) of the respective soil depths from each trial were analyzed for residues of bentazone and N-methylbentazone. In all cases the concentration of bentazone and N-methylbentazone in the unfortified control samples was below the LOQ, indicating the absence of interferences. Procedural recovery experiments performed with untreated field soil specimens spiked with bentazone and N-methylbentazone at concentration levels of 0.01, 0.1, 1.0, 2.0 and 12 mg/kg. The average recoveries for bentazone were in the range of 83 to 101% with relative standard deviations of 2.2 to 8.9% considering all four trials and all fortification levels. The average recoveries for N-methylbentazone over all four trials and fortification levels ranged from 93 to 105% with RSD's from 1.1 to 9.1%. These data prove that the analytical method applied was able to accurately determine bentazone and N-methylbentazone residues in soil samples of the sites down to a concentration of 0.01 mg/kg.

All residue values were related to the dry weight of the soil and were not corrected for procedural recoveries. Residue levels in mg/kg dry soil were converted to residue rates in g/ha taking into account the actual dry soil density of the field sample and were summed up for all depths between 0 and 50 cm analyzed. Results are presented in Table 7.3/14. The comprehensive analytical data (in mg/kg dry soil) including residues in the individual soil layers are presented in detail in the summary tables of Document L II, 7.3.

Table 7.3/14 Total residues of bentazone under field conditions in soil calculated to g/ha based on actual dry soil density and summed up for all depths analyzed

Trial Country	L110237 Goch-Nierswalde, Germany Replicate A	L110237 Goch-Nierswalde, Germany Replicate B	L110237 Goch-Nierswalde, Germany Replicate C		L110238 Stotzheim Northern France, Replicate A	L110238 Stotzheim Northern France, Replicate B	L110238 Stotzheim Northern France, Replicate C
Days after treatment	**[g/ha]**	**[g/ha]**	**[g/ha]**	**Days after treatment**	**[g/ha]**	**[g/ha]**	**[g/ha]**
0	1055*	835*	878*	**0**	749*	888*	950*
1	912	876	694	**1**	613	625	604
3	638	665	782	**3**	666	556	859
7	682	553	472	**8**	441	488	422
14	298	309	271	**16**	287	148	362
30	68	170	128	**30**	142	109	103
55	66	26	37	**57**	0	0	0
107	0	0	0	**91**	0	0	0
127	0	0	0	**115**	0	0	0
Trial Country	**L110239 Utrera, Spain Replicate A**	**L110239 Utrera, Spain Replicate B**	**L110239 Utrera, Spain Replicate C**		**L110240 Barry d' Islemade Southern France, Replicate A**	**L110240 Barry d' Islemade Southern France, Replicate B**	**L110240 Barry d' Islemade Southern France, Replicate C**
Days after treatment	**[g/ha]**	**[g/ha]**	**[g/ha]**	**Days after treatment**	**[g/ha]**	**[g/ha]**	**[g/ha]**
0	657*	646*	703*	**0**	712*	750*	749*
1	468	572	473	**1**	384	482	604
3	492	647	543	**3**	447	457	473
6	487	449	547	**7**	245	297	260
14	487	312	518	**14**	124	80	110
27	241	234	584	**30**	33	35	98
61	86	0	55	**56**	0	0	0
116	0	0	0	**94**	0	0	0
-	-	-	-	**127**	0	0	0

* Mean of two samples

Field soil samples taken from different depths were analyzed to a maximum of 127 days after treatment. Depth increments were analyzed at each sampling interval until two residue-free layers were reached. Beyond that, no samples were analyzed. Quantifiable levels (> 0.01 mg/kg) of bentazone were found to a depth of only 10 cm and until 55 DAT at Goch-Nierswalde trial, to a maximum of 30 cm and until 30 DAT at Stotzheim trial, to a maximum of 40 cm and 61 DAT at Utrera and to 20 cm and 30 DAT at Barry d´Islemade.

No residues above the LOQ could be detected in the 40-50 cm segment.

Metabolite N-methylbentazone could not be detected in three of the four trials. Only in trial L110240, the compound appeared at very low concentrations slightly above the LOQ (max. 0.018 mg/kg), and only in the 0-10 cm segment.

A detailed kinetic evaluation of the field trials is presented in a separate modeling report (see M II, 7.3.1/4)

Storage stability

In storage stability experiments an average recovery of 81 % of the initial bentazone was obtained after 24 months of storage in frozen soil (<-5°C) indicating that the compound is stable during the period investigated.

Preliminary experiments from the ongoing second stability study indicate that both compounds are stable in frozen soil for at least 2 years.

Shipping verification samples

Considering the analysis of the shipping verification samples with respect to bentazone, the concentrations ranged between 92 and 107 % of the initial amount. These results confirm that bentazone is stable during storage and shipment for at least 130 days.

III. Conclusions

Degradation of bentazone in soil could be followed applying a sensitive LC-MS/MS method with an appropriate LOQ of 0.01 mg/kg.

The analytical method used was shown to be applicable to correctly determine residues of bentazone and N-methylbentazone in soil. This was demonstrated by the absence of interferences in untreated soil as well as excellent procedural recovery rates with very low relative standard deviations in field and Li 10 soil.

Bentazone degraded in soil under field conditions at all four European sites down to a level with no residues above the LOQ (0.01 mg/kg) until the end of the study (nominal 120 days). DT_{50} values are supposed to be low and are the subject of a separate modeling report.

Residues of bentazone were found to a maximum depth of 30 cm and with only one exception in 40 cm. Metabolite N-methylbentazone was observed only at one trial site (L110239, Utrera, Spain), occurring sporadically and in low amounts (maximum of 0.018 mg/kg).

Application rates determined with application verification monitors and from day zero soil sampling are in good agreement.

Report:	II A 7.3.1/3 Eickler B., Budde E. 2012(a) Kinetic evaluation of six field dissipation trials with BAS 351 H - Bentazone conducted between 1989 and 1999: Determination of persistence and modeling endpoints according to FOCUS degradation kinetics BASF DocID 2011/1277148
Guidelines:	FOCUS Kinetics Report SANCO/10058/2005 ver. 2.0; EFSA Guidance to obtain DT50 values in soil (2010)
Testing Laboratory and dates:	BASF SE; Limburgerhof; Germany Fed.Rep. 17-Jan-2012 - 17-Jan-2012
GLP:	No, not subject to GLP regulations

Executive Summary

Kinetic evaluation of three field dissipation studies with BAS 351 H - bentazone was conducted in order to derive persistence and modelling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics and the EFSA guidance to obtain $DegT_{50}$ values in soil. The field studies comprise six field trials situated in different regions of Germany (five study sites) and one site in France, considering a range of different soils and climatic conditions.

In a first step kinetic evaluation of bentazone was performed with non-normalized data in order to derive degradation parameters that are valid as trigger endpoints. Kinetic evaluation showed that the dissipation behaviour of bentazone was best described using single first-order (SFO) kinetics. The non-normalized field half-lives (DT_{50}) for bentazone ranged between 7.4 to 23.8 days.

To derive field half-lives appropriate for environmental fate modelling, data derived from the field trials were normalized to reference conditions (20 °C, pF2) prior to kinetic evaluation by time-step normalization. Kinetic evaluation was performed on the normalized dataset and under consideration of the EFSA guidance (2010). The normalized (20 °C, pF2) field half-lives (DT_{50}) for bentazone ranged from 4.4 to 13.8 days.

I. MATERIAL AND METHODS

The kinetic evaluation was conducted for six field trials with bentazone from three field dissipation studies Schepers and Hesse, 1991 (1991/11611); Hesse and Schepers, 1991 (1991/11761) and Richter, 2000 (2000/1000137). The trials were situated in different regions of Germany (Goch-Nierswalde, Havixbeck, Limburgerhof, Holzen, Stetten) and one site in France (Le Pouliou), considering a range of different soils and climatic conditions (see Table 7.3/15). The individual field trials are briefly described in the original evaluation report.

Table 7.3/15 Locations of the different field trials

Trial No.	Country	Region	Location
H/A07/89	Germany	North Rhine-Westphalia	47574 Goch-Nierswalde
D08/028	Germany	North Rhine-Westphalia	48329 Havixbeck
VBL/156	Germany	Rhineland-Palatinate	67117 Limburgerhof
D07/94	Germany	Bavaria	84051 Holzen
VTU/81	Germany	Baden-Württemberg	74193 Stetten a. H.
Plot 602	France	Brittany, Côtes-d'Armor	Roc'h ar Vez; Le Pouliou

In the field trials conducted in Germany, bentazone was applied with a knapsack sprayer to bare soil at each study site at a nominal application rate of 1440 g a.s. ha^{-1}. Soil samples were taken at seven sampling dates up to 181 days after application. At each trial site, soil core replicates were taken up to a soil depth of 100 cm, divided into several segments, the soil combined for each layer and stored deep-frozen until analysis.

The soil samples were worked up and extracts analysed for bentazone by chromatographic analysis (GC/N-FID). The limit of quantification was 0.02 mg kg^{-1} and 0.01 mg kg^{-1} in study DE/H/A03/89 and DE/HA/011/90, respectively.

In the field trial conducted in France, bentazone was applied with a boom sprayer to maize at the 6 leaf growth stadium at a nominal application rate of 1200 g a.s. ha^{-1}. On average, the applied amount determined by field sample analysis in the top 10 cm of the soil cores right after application was 1575 g a.s. ha^{-1}, and soil filled Petri dish verification yielded 1103 g a.s. ha^{-1}. Soil samples were taken at nine sampling dates up to 57 days after application. At each sampling, core samplings down to a maximum soil depth of 50 cm were taken at 20 locations distributed over the plot along four parallel contour lines with five sampling points per line. The soil cores were divided into segments and stored deep-frozen until analysis.
The soil samples were worked up and extracts analysed for bentazone by chromatographic analysis (GC/MS). The limit of quantification was 0.02 mg kg^{-1}.

At all trial sites, bentazone residues were found mainly in the upper soil layer (0-10 cm to 0-15 cm). Residues above LOQ in deeper soil layers (15-37 cm) were detected only sporadically. After approximately two to three months, less than 10 % of the initial concentration of bentazone was reached at each of the six study sites.

Kinetic modelling

The software package KinGUI version 1.1 was used for parameter fitting [*SCHÄFER, D., MIKOLASCH, M., RAINBIRD, P., HARVEY, B. (2007) KinGUI: A new kinetic software tool for evaluations according to FOCUS Degradation Kinetics. BASF DocID 2007/1062781*]. The error tolerance and the number of iterations of the optimization tool were set to 0.00001 and 100, respectively.

- Values below the quantification or detection limit were treated as recommended by the FOCUS workgroup [see *FOCUS (2006): "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp., chapter 6.1.4 and chapter 8.3.1*]. The limit of quantification (LOQ) for bentazone reported in the three studies was 0.02 mg kg^{-1} [*Schepers, U., Hesse, B. (1991), Richter, T. (2000)*] and 0.01 mg kg^{-1} [*Hesse, B., Schepers, U. (1991)*]. LOD cannot be extracted from the study report and is therefore defined as LOQ. According to FOCUS, values below LOD were set to $0.5 \times$ LOD=0.01 mg kg^{-1} and 0.005 mg kg^{-1}, respectively.

- For each sampling point, total residues in the sampled soil core were calculated as sum of residues of the single soil core segments considering the height of the respective segment and assuming a soil bulk density of 1500 kg m^{-3}.

The measured data as well as resulting datasets submitted to kinetic analysis are provided in the original evaluation report.

Normalization procedure

Temperature correction factors (f_{temp}) were determined to account for differences between actual daily temperatures as calculated by FOCUS-PEARL 4.4.4 and a reference temperature of 20 °C using the Q_{10} approach as described in the report of the FOCUS soil modelling working group [see *FOCUS (1997): Soil persistence models and EU registration. The final report of the work of the Soil Modelling Work group of FOCUS (FOrum for the Co-ordination of pesticide fate models and their Use)*]. The Q_{10} response function was applied for temperatures above 0 °C (see Equation 1c). Below field temperatures of 0 °C it was assumed that no degradation occurs (Equation 1c). For the evaluation, the EFSA opinion on the default Q_{10} value [see *EFSA (2007): Scientific Opinion of the Panel on Plant Protection Products and their Residues on a request from EFSA related to the default Q10 value used to describe the temperature effect on transformation rates of pesticides in soil. The EFSA Journal (2007) 622, 1-32.*] was followed and a Q_{10} value of 2.58 was included in the assessment.

Moisture correction factors (f_{moist}) were determined to account for differences between actual daily soil moisture as calculated by FOCUS-PEARL 4.4.4 and the reference soil moisture (θ_{ref}) (Equation 1 d).

The normalized day lengths were derived according to Equation 1a. For DAT0, no normalization was considered and application was assumed to occur at the time point zero. Normalized sampling days (DAT_{norm}) after application were calculated by cumulatively summing up normalized day lengths according to Equation 1 b.

Equation 1 **Calculation of normalized day length based on combination of soil moisture and soil temperature correction factors**

a) $$D_{norm} = D * f_{temp} * f_{moisture}$$

b) $$t_i = \sum_{t=1}^{i-1} D_{norm}$$

with:
t_i = Time from application till sampling at day i [d]
D_{norm} = Normalized day length (20°C, pF2) [d]
i = Time span between application and sampling [d]

c) $$f_{temp} = \begin{cases} Q_{10}^{\frac{Tact-Tref}{10}} & \text{for } T_{act} > 0°C \\ 0 & \text{for } T_{act} \leq 0°C \end{cases}$$

d) $$f_{moist} = \begin{cases} (\frac{\theta_{act}}{\theta_{ref}})^B & \text{for } \theta_{ref} > \theta_{act} \\ 1 & \text{for } \theta_{ref} \leq \theta_{act} \end{cases}$$

with:
D_{norm} = normalized day length (temperature and moisture)
f_{temp} = temperature correction factor (-)
f_{moist} = moisture correction factor (-)
D = 1 d (days)
T_{act} = actual soil temperature (°C)
T_{ref} = reference temperature (20 °C)
Q_{10} = factor of increase of degradation rate with an increase in temperature of 10°C (Q_{10} = 2.58, EFSA opinion (-)
θ_{act} = actual soil moisture (vol. water content) (m^3 m^{-3})
θ_{ref} = reference soil moisture at pF2 (m^3 m^{-3})
B = exponent of the moisture response function, B = 0.7 (-)

Table 7.3/16 shows the field sampling dates for the trial locations and the normalized (20 °C, pF2) day lengths based on soil moisture and soil temperature data as simulated by FOCUS-PEARL 4.4.4.

Table 7.3/16 Time-step normalized (20 °C, pF2) sampling days

H/A07/89		D08/028		VBL/156		D07/94		VTU/81		Plot 602	
DAT	D_{norm}	DAT	D_{norm}	DAT	D_{norm}	DAT	D_{norm}	DAT	D_{norm}	DAT	D_{norm}
0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
14	6.4	14	5.0	14	11.0	7	4.5	7	3.4	2	1.0
30	17.4	28	12.5	30	23.4	14	9.2	13	7.8	4	1.8
60	38.7	57	29.0	60	53.6	30	17.6	29	18.0	6	2.8
100	65.5	98	59.5	102	97.5	61	40.1	61	36.5	8	3.9
140	88.2	139	86.8	140	121.1	104	78.0	103	75.5	10	5.0
180	103.1	181	109.2	183	137.5	131	96.7	134	101.7	14	7.0
										30	16.4
										57	30.6

DAT = days after treatment
D_{norm} = normalized day (20°C, pF2)

Kinetic evaluation to derive degradation parameters that are valid as trigger endpoints

For the non-normalized datasets the appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS kinetics guidance [*FOCUS (2006)*]. The best-fit model was selected based on visual and statistical assessment, and corresponding DT_{50} values are reported as trigger endpoints.

Kinetic evaluation to derive degradation parameters that are valid as modelling endpoints

Prior to kinetic evaluation, data derived from the field trials were normalized to reference conditions (20 °C, pF2) as described above. As given in EFSA [*EFSA (2010): EFSA Panel on Plant Protection Products; Guidance for evaluating laboratory and field dissipation studies to obtain DT50 values of plant protection products in soil. EFSA Journal 2010;8(12):1936, 67 pp.*], to guarantee that the residues describe the degradation in the soil matrix rather than at the soil surface, the Panel proposed the splitting of field dissipation studies into two parts viz. before and after at least 10 mm of rain has fallen since application. Thus, the field studies were assessed in regard to appropriate number of data points for a kinetic assessment if samplings before 10 mm of cumulative precipitation were excluded.

In the kinetic assessment for bentazone, the appropriate kinetic model was selected in a step-wise approach proposed by the EFSA guidance [*EFSA (2010)*] to derive the appropriate endpoints from each field dissipation trial. The goodness-of-fit was assessed based on visual and statistical assessment, and corresponding DT_{50} values are reported as modelling endpoints.

Kinetic models included in the evaluations

The kinetic models which can be employed for these evaluations were described by FOCUS workgroup on degradation kinetics [*FOCUS (2006)*]:

- Single-First-Order (SFO); Box 5-1, p. 51 in *FOCUS (2006)*,
- Gustafson and Holden (FOMC): Box 5-2, p. 53 in *FOCUS (2006)*
- Hockey-stick (HS) kinetic: Box 5-3, p. 55 in *FOCUS (2006)*
- Bi-exponential (DFOP) kinetic: Box 5-4, p. 57 in *FOCUS (2006)*

According to the EFSA guidance [*EFSA (2010)*], the kinetic models named above with exception of the FOMC model are proposed for the calculation of the DT_{50} for normalized decline curves.

The appropriateness of a distinct kinetic model to describe soil degradation can be tested according to the following criteria recommended by FOCUS [*FOCUS (2006)*, chapter 6.3.1]:

- Visual assessment of goodness-of-fit
- Statistical evaluation of the goodness-of-fit by estimation of the error percentage at which the χ^2 test is passed (Equation 6-2; p. 89 in *FOCUS (2006)*)
- Estimation of the reliability of parameter estimation using the t-test to evaluate whether estimated degradation parameters differ significantly from zero (Equation 6-3; p. 93 in *FOCUS (2006)*)

A kinetic model is considered appropriate if the residuals are randomly distributed around zero, the χ^2 error value is <15 % and the estimated degradation parameters differ from zero as outlined by FOCUS [*FOCUS (2006)*, chapter 6.3.1].

II. RESULTS AND DISCUSSION

Results of the kinetic assessment for persistence endpoints

The dissipation behaviour of bentazone in six field trials was analysed following the recommended procedure to derive endpoints for parent compounds and metabolites presented in FOCUS degradation kinetics [*FOCUS (2006)*].

Kinetic evaluation showed that the dissipation behaviour of bentazone was best described using single first-order (SFO) kinetics. A summary of the adequate rate constants (*k*) and DT_{50} and DT_{90} values to be used as persistence endpoints is given in Table 7.3/17.

Table 7.3/17 Summary of persistence endpoints of bentazone

Field trial	Soil type* (DIN)	Best-fit kinetic model	Rate k [d^{-1}]	DT_{50} [d]	DT_{90} [d]
Goch-Nierswalde, Germany, H/A07/89	Clayey silt	SFO	0.0458	15.1	50.3
Havixbeck, Germany, D08/028	Loamy-sandy silt	SFO	0.0523	13.3	44.0
Limburgerhof, Germany, VBL/156	Loamy sand	SFO	0.0717	9.7	32.1
Holzen, Germany, D07/94	Sandy loam	SFO	0.0291	23.8	79.0
Stetten a. H., Germany, VTU/81	Clayey loam	SFO	0.0385	18.0	59.9
Le Pouliou, France, Plot 602	Loamy sand	SFO	0.0941	7.4	24.5
* German Classification (DIN 4220), as USDA characteristics were not available					

Results of the kinetic assessment for modelling endpoints

In the kinetic assessment for modelling endpoints for bentazone, the appropriate kinetic model was selected in a step-wise approach proposed by the EFSA guidance [*EFSA (2010)*].

The rate constants (*k*) and field half-lives (DT_{50}) adequate to be used in environmental fate modelling are summarized in Table 7.3/18.

Table 7.3/18 Summary of modelling endpoints of bentazone

Field trial	Soil type (DIN)	Kinetic model	All data included		Data > 10 mm rainfall only	
			Rate k [d^{-1}]	DT_{50} [d]	Rate k [d^{-1}]	DT_{50} [d]
Goch-Nierswalde, Germany, H/A07/89	Clayey silt	SFO	0.0862	8.0	0.0729	9.5
Havixbeck, Germany, D08/028	Loamy-sandy silt	SFO	0.1305	5.3	0.1575	4.4
Limburgerhof, Germany, VBL/156	Loamy sand	SFO	0.0918	7.6	not calculated *	
Holzen, Germany, D07/94	Sandy loam	SFO	0.0468	14.8	0.0577	12.0
Stetten a. H., Germany, VTU/81	Clayey loam	SFO	0.0625	11.1	0.0504	13.8
Le Pouliou, France, Plot 602	Loamy sand	SFO	0.1904	3.6	0.1032	6.7
Geometric mean			**0.0912**	**7.6**	**0.0808**	**8.6**
*calculation was not conducted due to insufficient number of data points (n=3)						

III. CONCLUSION

The dissipation behaviour of BAS 351 H – bentazone in soil has been investigated in three field dissipation studies including six field trials. The purpose of this evaluation was to analyze the degradation kinetics of BAS 351 H – bentazone observed in the six soils according to the current guidance of the FOCUS workgroup on degradation kinetics and the EFSA guidance to obtain $DegT_{50}$ values in soil.

Kinetic evaluation showed that the dissipation behaviour of bentazone was best described using single first-order (SFO) kinetics. The non-normalized field half-lives (DT_{50}) for bentazone ranged from 7.4 to 23.8 days. The normalized (20 °C, pF2) field half-lives (DT_{50}) for bentazone ranged from 4.4 to 13.8 days.

Report:	II A 7.3.1/4 Budde E., Matejek B. 2012(a) Kinetic evaluation of four field dissipation trials with BAS 351 H - Bentazone conducted in 2008: Determination of persistence and modeling endpoints according to FOCUS degradation kinetics BASF DocID 2011/1277149
Guidelines:	FOCUS Kinetics Report SANCO/10058/2005 ver. 2.0; EFSA Guidance to obtain DT50 values in soil (2010)
Testing Laboratory and dates:	BASF SE; Limburgerhof; Germany Fed.Rep. 17-Jan-2012 - 17-Jan-2012
GLP:	No, not subject to GLP regulations

Executive Summary

Kinetic evaluation of a field dissipation study with BAS 351 H - bentazone was conducted in order to derive persistence and modelling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics and the EFSA guidance to obtain $DegT_{50}$ values in soil. The field study comprises four field trials at four different locations in Europe with different soils and climate conditions.

In a first step kinetic evaluation of bentazone was performed with non-normalized data in order to derive field dissipation parameters that are adequate to be used as persistence endpoints. Kinetic evaluation showed that the dissipation behaviour of bentazone was best described using single first-order (SFO) kinetics. The non-normalized field half-lives (DT_{50}) for bentazone ranged from 3.9 to 8.9 days.

To derive field half-lives appropriate for environmental fate modelling, data derived from two out of the four field trials were normalized to reference conditions (20 °C, pF2) prior to kinetic evaluation by time-step normalization. Kinetic evaluation was performed on the normalized dataset and under consideration of the EFSA guidance (2010). The kinetic evaluation showed that the field decline of bentazone could be well described with SFO kinetics in the two trials. The normalized (20 °C, pF2) field half-lives (DT_{50}) for bentazone ranged from 6.9 to 12.2 days.

The metabolite N-methyl-bentazone could not be detected in any of the soil samples and was therefore not considered in the kinetic evaluation.

I. MATERIAL AND METHODS

The kinetic evaluation was conducted for four field trials with bentazone from one field dissipation study [see II A 7.3.1/1, Richter and Kuhnke, 2012, 2010/1151827]. The trials were situated in different regions of Germany (two study sites), one site in France and one site in Italy, considering a range of different soils and climatic conditions (see Table 7.3/19). The individual field trials are briefly described in the original evaluation report.

Table 7.3/19 Locations of the different field trials

Trial No.	Country	Region	Location
L080157	Germany	Rhineland-Palatinate	Waldsee
L080158	Germany	North Rhine-Westphalia	Goch-Nierswalde
L080159	France	Midi-Pyénées	Meauzac
L080160	Italy	Emilia-Romagna	Poggio Renatico

The nominal application rate was 1000 g a.s. ha^{-1} at all trial sites. For application verification, 20 soil-filled Petri dishes were placed on the treated plot (five per subplot). Applications were made to bare soil and were conducted between mid-April and end of May 2008 using a calibrated boom sprayer. [see *II A 7.3.1/1*]. The mean application rates determined from Petri dish verifications ranged from 92.9% to 100.1% of the target rate.

Soil samples were taken at eight sampling dates up to 115 days after application and down to a soil depth of up to 50 cm (Waldsee, Germany 60 cm) from four individual subplots. Soil cores were cut into segments of 10 cm under ambient conditions directly after sampling. Soil segments of the same depth were pooled and homogenized and then stored deep frozen at approximately -18°C until analysis of representative sub-samples of each depth. Samples from all field trials were worked up and extracts analysed for bentazone by chromatographic analysis ((UPLC)-MS/MS). The limit of quantification was 0.01 mg kg^{-1}.

Kinetic modelling

The software package KinGUII [*Meyer, H. (2011): KinGUII -Manual for the Graphical User Interface. Bayer Crop Science AG. 22pp.*] was used for parameter fitting. Datasets were prepared for kinetic evaluation as follows.

- Values below the quantification or detection limit were treated as recommended by the FOCUS workgroup [see *FOCUS (2006), chapter 6.1.4*]. The limit of quantification (LOQ) reported in the original study report was 0.01 mg kg^{-1} [see *II A 7.3.1/2*]. The limit of detection (LOD) could not be extracted from the study report and was therefore defined as LOQ. According to FOCUS, values below LOD were set to 0.5 × LOD = 0.005 mg kg^{-1}.

- Following the steps described above, the soil residues in mg kg^{-1} dry soil were converted to a mass per area basis (g ha^{-1}), taking into account actual dry soil bulk densities of the field samples and the corresponding depth increment.

The measured data as well as resulting datasets submitted to kinetic analysis are provided in the original evaluation report.

Normalization procedure

Temperature correction factors (f_{temp}) were determined to account for differences between actual daily temperatures as calculated by FOCUS-PEARL 4.4.4 and a reference temperature of 20 °C using the Q_{10} approach as described in the report of the FOCUS soil modelling working group [see *FOCUS (1997)*]. The Q_{10} response function was applied for temperatures above 0 °C (see description under *II A 7.3.1/3*, Equation 1c). Below field temperatures of 0 °C it was assumed that no degradation occurs (Equation 1c). For the evaluation, the EFSA opinion on the default Q_{10} value [see *EFSA (2007)*] was followed and a Q_{10} value of 2.58 was included in the assessment.

Moisture correction factors (f_{moist}) were determined to account for differences between actual daily soil moisture as calculated by FOCUS-PEARL 4.4.4 and the reference soil moisture (θ_{ref}) (see description under *II A 7.3.1/3,* Equation 1d).

The normalized day lengths were derived as described under *II A 7.3.1/3* (Equation 1a). For DAT0, no normalization was considered and application was assumed to occur at the time point zero. Normalized sampling days (DAT_{norm}) after application were calculated by cumulatively summing up normalized day lengths as described under *II A 7.3.1/3* Equation 1b).

Table 7.3/20 shows the field sampling dates for the two out of four trial locations that were considered for evaluation according to EFSA guidance [*EFSA (2010)*] and the normalized (20 °C, pF2) day lengths based on soil moisture and soil temperature data as simulated by FOCUS-PEARL 4.4.4.

Table 7.3/20 Time-step normalized (20 °C, pF2) sampling days

Waldsee (L080157)		Poggio Renatico (L080160)	
DAT	**D_{norm}**	**DAT**	**D_{norm}**
0	0	0	0
1	0.8	1	0.5
3	2.5	3	1.5
6	4.9	7	3.5
14	11.0	16	9.5
27	17.1	34	26.5
65	44.7	63	67.3
113	85.8	115	140.1

DAT = days after treatment
D_{norm} = normalized day (20°C, pF2)

Kinetic evaluation to derive degradation parameters that are valid as trigger endpoints

The appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS kinetics guidance [*FOCUS (2006)*]. The best-fit model was selected based on visual and statistical assessment, and corresponding DT_{50} values are reported as trigger endpoints.

Kinetic evaluation to derive degradation parameters that are valid as modelling endpoints

To derive appropriate field half-lives for environmental fate modelling the recommendations provided in the EFSA guidance to obtain $DegT_{50}$ values in soil were considered [*EFSA (2010)*], i.e. splitting of field dissipation trials into two parts viz. before and after at least 10 mm of rain has fallen since application. The field studies were assessed in regard to an appropriate number of data points for a kinetic assessment if samplings before 10 mm of cumulative precipitation were excluded. Four to five data points remained for the kinetic evaluation of the field trials L080157 and L080160. For the field trials L080158 and L080159, only three data points with residue concentrations >LOD remained after consideration of >10 mm cumulative precipitation (Table 7.3/21). They were therefore excluded from further evaluation according to EFSA.

Table 7.3/21 Summary of evaluable field trials according to EFSA (2010)

Trial	Location	Weather station	Day of 10 mm rain (DAT)	Amount of rain until 1st considered data point [mm]	Remaining data points
L080157	Waldsee	Speyer	5	10	5
L080158	Goch-Nierswalde	n/a	13	23.4	3
L080159	Meauzac	n/a	4	26	3
L080160	Poggio Renatico	on-site	12	71.1	4

Prior to kinetic evaluation, data derived from the two remaining field trials were normalized to reference conditions (20 °C, pF2) as described above. Kinetic evaluation was then performed on the normalized dataset. In the kinetic assessment for bentazone, the appropriate kinetic model was selected in a step-wise approach proposed by the EFSA guidance [*EFSA (2010)*] to derive the appropriate endpoints from each field dissipation trial. The goodness-of-fit was assessed based on visual and statistical assessment, and corresponding DT_{50} values are reported as modelling endpoints.

Kinetic models included in the evaluations

The kinetic models employed for the evaluations are listed under *II A 7.3.1/3.* The appropriateness of a distinct kinetic model to describe soil degradation can be tested as described under *II A 7.3.1/3.* A kinetic model is considered appropriate if the residuals are randomly distributed around zero, the χ^2 error value is <15 % and the estimated degradation parameters differ from zero as outlined by FOCUS [*FOCUS (2006)*]

II. RESULTS AND DISCUSSION

Results of the kinetic assessment for persistence endpoints

The dissipation behaviour of bentazone in four field trials was analysed following the recommended procedure to derive endpoints for parent compounds presented in FOCUS degradation kinetics [*FOCUS (2006)*].

Kinetic evaluation showed that the dissipation behaviour of bentazone was best described using single first-order (SFO) kinetics. A summary of the adequate rate constants (*k*) and DT_{50} and DT_{90} values to be used as persistence endpoints is given in Table 7.3/22.

Table 7.3/22 Summary of persistence endpoints of bentazone derived from European field dissipation trials

Field trial (Trial no)	Best-fit kinetic model	Rate k [d^{-1}]	DT_{50} [d]	DT_{90} [d]
Waldsee, Germany (L080157)	SFO	0.0782	8.9	29.4
Goch-Nierswalde, Germany (L080158)	SFO	0.1226	5.7	18.8
Meauzac, France (L080159)	SFO	0.1778	3.9	12.9
Poggio Renatico, Italy (L080160)	SFO	0.1039	6.7	22.2

Results of the kinetic assessment for modelling endpoints

The field studies were first assessed in regard to an appropriate number of data points for a kinetic assessment if samplings before 10 mm of cumulative precipitation were excluded.

The dissipation behaviour of bentazone in the two remaining field trials was then analysed in a step-wise approach proposed by the EFSA guidance [*EFSA (2010)*].

The rate constants (*k*) and field half-lives (DT_{50}) adequate to be used in environmental fate modelling are summarized in Table 7.3/23.

Table 7.3/23 Summary of modelling endpoints of bentazone derived from European field dissipation trials

Field trial (Trial no)	Kinetic model	All data included		Data > 10 mm rainfall only	
		Rate k [d^{-1}]	DT_{50} [d]	Rate k [d^{-1}]	DT_{50} [d]
Waldsee, Germany (L080157)	SFO	0.1047	6.6	0.1005	6.9
Poggio Renatico, Italy (L080160)	SFO	0.1957	3.5	0.0571	12.2

III. CONCLUSION

The dissipation behaviour of bentazone in soil has been investigated in a field dissipation study including four field trials at four different locations in Europe. The purpose of this evaluation was to analyze the dissipation behaviour of bentazone in four soils under different climatic conditions and to derive persistence and modelling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics and the EFSA guidance to obtain $DegT_{50}$ values in soil.

Kinetic evaluation showed that the dissipation behaviour of bentazone was best described using single first-order (SFO) kinetics. The non-normalized field half-lives (DT_{50}) for bentazone ranged from 3.9 to 8.9 days. The normalized (20 °C, pF2) field half-lives (DT_{50}) for bentazone ranged from 6.9 to 12.2 days.

Report:	II A 7.3.1/5 Matejek B., Budde E. 2012(b) Kinetic evaluation of four field dissipation trials with BAS 351 H - Bentazone conducted in 2011: Determination of persistence and modeling endpoints according to FOCUS degradation kinetics BASF DocID 2011/1277150
Guidelines:	FOCUS Kinetics Report SANCO/10058/2005 ver. 2.0; EFSA Guidance to obtain DT50 values in soil (2010)
Testing Laboratory and dates:	BASF SE; Limburgerhof; Germany Fed.Rep. 17-Jan-2012 - 17-Jan-2012
GLP:	No, not subject to GLP regulations

Executive Summary

Kinetic evaluation of a field dissipation study with BAS 351 H - bentazone was conducted in order to derive persistence and modelling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics and the EFSA guidance to obtain $DegT_{50}$ values in soil. The field study comprises four field trials at four different locations in Europe with four different soils under different climate conditions.

In a first step kinetic evaluation of bentazone was performed with non-normalized data in order to derive field dissipation parameters that are adequate to be used as persistence endpoints. Kinetic evaluation showed that the dissipation behaviour of bentazone was best described using single first-order (SFO) kinetics. The non-normalized field half-lives (DT_{50}) for bentazone ranged from 5.4 to 26.4 days.

To derive field half-lives appropriate for environmental fate modelling, data derived from the field trials were normalized to reference conditions (20 °C, pF2) prior to kinetic evaluation by time-step normalization. Kinetic evaluation was performed on the normalized dataset and under consideration of the EFSA guidance (2010). The kinetic evaluation showed that the field decline of bentazone could be well described with SFO kinetics in the four trials. The normalized (20 °C, pF2) field half-lives (DT_{50}) for bentazone ranged from 3.0 to 31.3 days.

The metabolite N-methyl-bentazone was observed only at the Spanish test site (L110239), occurring sporadically and in low amounts (< 5% of the applied parent amount). The metabolite was therefore not considered in the kinetic evaluation.

I. MATERIAL AND METHODS

The kinetic evaluation was conducted for four field trials with bentazone from one field dissipation study [see II A 7.3.1/2, Richter T. 2012, 2011/1277036]. The four field trials were situated in different regions of Europe with two test sites in France (Stotzheim, Barry d'Islemade), one site in Germany (Goch-Nierswalde) and one site in Spain (Utrera), covering a range of different soils and climatic conditions (see Table 7.3/24). The individual field trials are briefly described in the original evaluation report.

Table 7.3/24 Locations of the different field trials

Trial No.	Country	Region	Location
L110237	Germany	North Rhine-Westphalia	47574 Goch-Nierswalde
L110238	France	Alsace	67140 Stotzheim
L110239	Spain	Andalusia	41710 Utrera
L110240	France	Midi-Pyrénées	82290 Barry d'Islemade

The formulation BAS 351 32 H (480.0 g/L BAS 351 H, SL) was applied once as spray application at a nominal application rate equivalent to 1000 g a.i./ha of bentazone. The trial areas were divided into two plots: one plot was used as untreated control the other was treated with the test item and considered of three subplots that were assigned for replicates. For application verification, soil-filled Petri dishes were distributed over the three replicates right before the application (10 per replicate) and collected immediately after the application [see *II A 7.3.1/2*]. The mean application rates determined from Petri dish verifications ranged from 99.6% to 109.3% of the target rate. Applications were made to bare soil and were conducted between April and end of May 2011 using a calibrated boom sprayer [see *II A 7.3.1/2*]. After application and sampling of Petri dishes, the whole area was irrigated in order to allow the compound to percolate off the surface to prevent any surface dissipation processes, e.g. photolysis.

Soil samples were taken prior and up to 127 days after application and down to a soil depth of 50 cm. Soil cores were cut into segments of 10 cm under ambient conditions directly after sampling. Soil segments of the same depth were pooled and homogenized, and a representative sub-sample of each depth was taken for residue analysis. The samples were stored deep frozen at -18 °C or below prior until analysis.
Samples from all field trials were worked up and extracts analysed for bentazone by chromatographic analysis (LC-MS/MS). The limit of quantification was 0.01 mg kg^{-1}[see *II A 7.3.1/2*].

Kinetic modelling

The software package KinGUII [*Meyer, H. (2011): KinGUII - Manual for the Graphical User Interface. Bayer Crop Science AG. 22pp.*] was used for parameter fitting. Datasets were prepared for kinetic evaluation as follows.

- Values below the quantification or detection limit were treated as recommended by the FOCUS workgroup [see *FOCUS (2006), chapter 6.1.4 and chapter 8.3.1*]. The limit of quantification (LOQ) reported in the original study report was 0.01 mg kg^{-1} [see *II A 7.3.1/2*]. The limit of detection (LOD) could not be extracted from the study report and was therefore defined as LOQ. According to FOCUS, values below LOD were set to 0.5 × LOD = 0.005 mg kg^{-1}.

- Following the steps described above, the soil residues in mg kg^{-1} dry soil were converted to a mass per area basis (g ha^{-1}), taking into account actual dry soil bulk densities of the field samples and the corresponding depth increment.

The measured data as well as resulting datasets submitted to kinetic analysis are provided in the original evaluation report.

Normalization procedure

Temperature correction factors (f_{temp}) were determined to account for differences between actual daily temperatures as calculated by FOCUS-PEARL 4.4.4 and a reference temperature of 20 °C using the Q_{10} approach as described in the report of the FOCUS soil modelling working group [see *FOCUS (1997)*]. The Q_{10} response function was applied for temperatures above 0 °C (see description under *II A 7.3.1/3*, Equation 1c). Below field temperatures of 0 °C it was assumed that no degradation occurs (see *II A 7.3.1/3,* Equation 1c). For the evaluation, the EFSA opinion on the default Q_{10} value [see *EFSA (2007)*] was followed and a Q_{10} value of 2.58 was included in the assessment.

Moisture correction factors (f_{moist}) were determined to account for differences between actual daily soil moisture as calculated by FOCUS-PEARL 4.4.4 and the reference soil moisture (θ_{ref}) (see description under *II A 7.3.1/3,* Equation 1d).

The normalized day lengths were derived as described under *II A 7.3.1/3* (Equation 1a). For DAT0, no normalization was considered and application was assumed to occur at the time point zero. Normalized sampling days (DAT_{norm}) after application were calculated by cumulatively summing up normalized day lengths as described under *II A 7.3.1/3* (Equation 1b).

Table 7.3/25 shows the field sampling dates for the trial locations and the normalized (20 °C, pF2) day lengths based on soil moisture and soil temperature data as simulated by FOCUS-PEARL 4.4.4.

Table 7.3/25 Time-step normalized (20 °C, pF2) sampling days

Goch-Nierswalde (L110237)		Stotzheim (L110238)		Utrera (L110239)		Barry d'Islemade (L110240)	
DAT	D_{norm}	DAT	D_{norm}	D_{norm}	DAT	D_{norm}	DAT
0	0	0	0	0	0	0	0
1	0.6	1	0.7	1	1.1	1	0.8
3	1.5	3	1.7	3	3.0	3	2.1
7	3.3	8	3.8	6	6.2	7	4.0
14	6.3	16	8.4	14	16.0	14	6.8
30	13.6	30	16.2	27	28.9	30	15.0
55	26.2	57	32.0	61	78.6	56	29.0
107	57.1	91	54.3	116	193.1	94	62.3
127	71.5	115	72.7	./.	./.	127	93.0

DAT = days after treatment
D_{norm} = normalized day (20°C, pF2)

Kinetic evaluation to derive degradation parameters that are valid as trigger endpoints

For the non-normalized datasets the appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS kinetics guidance [*FOCUS (2006)*]. The best-fit model was selected based on visual and statistical assessment, and corresponding DT_{50} values are reported as trigger endpoints.

Kinetic evaluation to derive degradation parameters that are valid as modelling endpoints

Prior to kinetic evaluation, data derived from the field trials were normalized to reference conditions (20 °C, pF2) as described above. As given in EFSA [*EFSA (2010)*], to guarantee that the residues describe the degradation in the soil matrix rather than at the soil surface, the Panel proposed the splitting of field dissipation studies into two parts viz. before and after at least 10 mm of rain has fallen since application. Since the trial sites were irrigated directly after application (≥ 10 mm) to allow the compound to percolate off the surface and to prevent any surface dissipation processes, all data points could be included in the kinetic assessment.

In the kinetic assessment for bentazone, the appropriate kinetic model was selected in a step-wise approach proposed by the EFSA guidance [*EFSA (2010)*] to derive the appropriate endpoints from each field dissipation trial. The goodness-of-fit was assessed based on visual and statistical assessment, and corresponding DT_{50} values are reported as modelling endpoints.

Kinetic models included in the evaluations

The kinetic models employed for the evaluations are listed under *II A 7.3.1/3.* The appropriateness of a distinct kinetic model to describe soil degradation can be tested as described under *II A 7.3.1/3.* A kinetic model is considered appropriate if the residuals are randomly distributed around zero, the χ^2 error value is <15 % and the estimated degradation parameters differ from zero as outlined by FOCUS [*FOCUS (2006)*]

II. RESULTS AND DISCUSSION

Results of the kinetic assessment for persistence endpoints

The dissipation behaviour of bentazone in four field trials was analysed following the recommended procedure to derive endpoints for parent compounds presented in FOCUS degradation kinetics [*FOCUS (2006)*].

Kinetic evaluation showed that the dissipation behaviour of bentazone was best described using single first-order (SFO) kinetics. A summary of the adequate rate constants (*k*) and DT_{50} and DT_{90} values to be used as persistence endpoints is given in Table 7.3/26.

Table 7.3/26 Summary of persistence endpoints of bentazone derived from European field dissipation trials

Field trial (Trial no)	Best-fit kinetic model	Rate k [d^{-1}]	DT_{50} [d]	DT_{90} [d]
Goch-Nierswalde, Germany (L110237)	SFO	0.0735	9.4	31.3
Stotzheim, France (L110238)	SFO	0.0659	10.5	34.9
Utrera, Spain (L110239)	SFO	0.0263	26.4	87.7
Barry d'Islemade, France (L110240)	SFO	0.1274	5.4	18.1

Results of the kinetic assessment for modelling endpoints

In the kinetic assessment for modelling endpoints for bentazone, the appropriate kinetic model was selected in a step-wise approach proposed by the EFSA guidance [*EFSA (2010)*]. Since directly after application the trial sites were irrigated to allow the compound to percolate off the surface and to prevent any surface dissipation processes all data points could be included in the kinetic assessment.

The rate constants (*k*) and field half-lives (DT_{50}) adequate to be used in environmental fate modelling are summarized in Table 7.3/27.

Table 7.3/27 Summary of modelling endpoints of bentazone derived from European field dissipation trials

Field trial (Trial no)	Kinetic model	Rate k [d^{-1}]	DT_{50} [d]
Goch-Nierswalde, Germany (L110237)	SFO	0.1625	4.3
Stotzheim, France (L110238)	SFO	0.1290	5.4
Utrera, Spain (L110239)	SFO	0.0222	31.3
Barry d'Islemade, France (L110240)	SFO	0.2349	3.0

III. CONCLUSION

The dissipation behaviour of bentazone in soil has been investigated in a field dissipation study including four field trials at four different locations in Europe. The purpose of this evaluation was to analyze the degradation kinetics of BAS 351 H – bentazone observed in the four soils according to the current guidance of the FOCUS workgroup on degradation kinetics and the EFSA guidance to obtain $DegT_{50}$ values in soil.

Kinetic evaluation showed that the dissipation behaviour of bentazone was best described using single first-order (SFO) kinetics. The non-normalized field half-lives (DT_{50}) for bentazone ranged from 5.4 to 26.4 days. The normalized (20 °C, pF2) field half-lives (DT_{50}) for bentazone ranged from 3.0 to 31.3 days.

Summary of all field dissipation half lives

A summary of all estimated persistence and modelling half-lives is given in Table 7.3/28. The half-lives from the older studies were included for the derivation of the relevant persistence and modelling endpoints. The half-lives used as persistence endpoints were in the range from 3.9 - 26.4 days. The maximum DT_{90} value calculated was 87.7 days. The half-lives used as modelling endpoints ranged from 3.0 - 31.3 days with a median of 6.9 days.

Table 7.3/28 Summary of soil dissipation studies and degradation rates of bentazone

Field trial	Soil type* (DIN)	Persistent endpoints			Modelling Endpoints		Ref. of soil study	Ref. of kin. evaluation
		Best-fit kinetic model	$DisT_{50}$ [d]	$DisT_{90}$ [d]	Kinetic model	$DegT_{50}$ [d]		
Goch-Nierswalde, Germany, H/A07/89	Clayey silt*	SFO	15.1	50.3	SFO	9.5	Schepers and Hesse, 1991 1991/11611	Eickler and Budde, 2011, 2011/1277148
Havixbeck, Germany, D08/028	Loamy-sandy silt*	SFO	13.3	44.0	SFO	4.4		
Limburgerhof, Germany, VBL/156	Loamy sand*	SFO	9.7	32.1	SFO	n.c. #		
Holzen, Germany, D07/94	Sandy loam*	SFO	23.8	79.0	SFO	12.0	Hesse and Schepers, 1991 1991/11761	
Stetten a. H., Germany, VTU/81	Clayey loam*	SFO	18.0	59.9	SFO	13.8		
Le Pouliou, France, Plot 602	Loamy sand*	SFO	7.4	24.5	SFO	6.7	Richter, 2000 2000/1000137	
Waldsee, Germany, L080157	loamy sand**	SFO	8.9	29.4	SFO	6.9	Richter and Kuhnke, 2012, 2010/1151827	Matejek and Budde, 2011(a), 2011/1277149
Goch-Nierswalde, Germany, L080158	silt loam**	SFO	5.7	18.8	SFO	n.c. #		
Meauzac, France, L080159	Loam**	SFO	3.9	12.9	SFO	n.c. #		
Poggio Renatico, Italy, L080160	Silt loam**	SFO	6.7	22.2	SFO	12.2		
Goch-Nierswalde, Germany, L110237	Silt loam**	SFO	9.4	31.3	SFO	4.3	Richter, 2012, 2011/1277036	Matejek and Budde, 2011(b), 2011/1277150
Stotzheim, France, L110238	Silt loam **	SFO	10.5	34.9	SFO	5.4		
Utrera, Spain, L110239	Sand**	SFO	26.4	87.7	SFO	31.3		
Barry d'Islemade, France, L110240	Sandy loam**	SFO	5.4	18.1	SFO	3.0		
Maximum value			**26.4**	**87.7**	**median**	**6.9**		

Only studies with ≥4 data points where included in the assessment. Sampling points before 10 mm of cumulative precipitation were not considered according to EFSA.

* German classification, ** USDA classification

7.3.2 Soil residue testing

No soil residue testing was performed. The field soil dissipation data provided in AII 7.3.1 are reliable for estimation of soil residues at the time when succeeding crops are grown.

7.3.3 Soil accumulation testing on relevant soils

All field soil dissipation DT_{90} values obtained for bentazone were clearly less than 1year. Therefore, soil accumulation studies are not considered necessary.

7.4 Mobility studies

7.4.1 Adsorption and desorption of the active substance

The mobility of bentazone in soil was evaluated during the former Annex I inclusion process. A brief review of the adsorption/desorption behaviour of bentazone based on the data considered within the former EU review process is provided below.

The EU review report lists K_{oc} values in the range of 13.2 to 175.6 mL g^{-1} (Table 7.4/1, line 1 to 8).

In addition to the studies already peer reviewed and listed in the EU review report of bentazone, three further studies had been included in the adsorption evaluation (Table 7.4/1, line 9 - 12). All existing national registrations of bentazone containing products within the EU since Annex I inclusion in 2000 were based on these 12 data sets.

The sorption of bentazone in soil had to be considered as low as measured in batch equilibrium experiments. The organic carbon related sorption coefficients of bentazone in soil ($K_{f,oc}$ values) ranged from 3.0 to 175.6 mL g^{-1}, with a median $K_{f,oc}$ value of 25.2 mL g^{-1}. The sorption isotherms showed a considerable non-linearity with a median Freundlich exponent of 0.85.

Table 7.4/1 Adsorption values of bentazone in different soils (used since 2000)

No.	Soil Origin	Soil Type	pH	Organic carbon [%]	1/n	$K_{f,oc}$ [mL g^{-1}]	Source
1	Pfungstadt	loam	7.3	0.58	0.99	37.1	EU review report
2	Neuhofen	loamy sand	7.2	2.66	1.03	13.3	EU review report
3	LUFA	sand	7.0	0.51	1.13	46.5	EU review report
4	Monticeller (IL)	clay	5.4	1.80	0.66	23.4	EU review report
5	Renvill (MI)	clay	7.7	2.91	0.70	13.2	EU review report
6	Briggs (CA)	heavy clay	4.3	1.74	0.70	175.6	EU review report
7	Pope Farm (NC)	loamy sand	5.0	0.58	0.69	77.6	EU review report
8	Greenville (MS)	clay sediment (rice soil)	6.6	0.70	0.56	25.2	EU review report
9	Mellby (Sweden)	sandy loam	6.2	5.90	0.80	49.2	#1994/10464 II A 7.4.1/1
10	Vredepeel (NL)	sand	5.2	3.00	0.97	6.4	#1995/10689 II A 7.4.1/2
11	Speyrer Wald	sand	6.0	0.70	0.85	3.0	#1999/10685 II A 7.4.1/3
12	Borstel	sandy loam	5.7	1.20	0.98	5.9	#1999/10685 II A 7.4.1/3
	Median				**0.85**	**25.2**	

Although the adsorption values of the latter three studies had been used for the leaching assessment of bentazone for some years, the studies had never been summarized according to OECD format or peer-reviewed on EU level. For sake of completeness, the summaries of those studies are therefore provided below.

Furthermore, during public literature search on the environmental behaviour of bentazone, several publications on the adsorption behaviour of bentazone were found. The publications were thoroughly evaluated on their suitability and quality to be implemented into the EU registration process, and overall some publications were considered to be of sufficient high quality and to provide valid and useful data to describe the adsorption behaviour of bentazone in agricultural soils. A summary of those publications is also provided below.

A detailed evaluation of all available sorption values according to newest EU guidance documents and derivation of the adsorption endpoint to be used in leaching assessment is then provided in M III A, chapter 9.6 PEC groundwater.

Report:	II A 7.4.1/1 Bergstroem L. et al. 1994(b) Pesticide leaching data to validate simulation models for registration purposes BASF DocID 1994/10464
Guidelines:	None
Testing Laboratory and dates:	01-Jan-1994
GLP:	No, not subject to GLP regulations (scientific publication)

Executive Summary

Bentazone sorption experiments were performed in one Swedish sandy loam soil with a pH of 6.2 and an organic matter content of 5.9 %. The investigation was done within a lysimeter study on four different soil types. Only the results of the sorption experiments are described in the following.

The sorption experiment was performed according to OECD guideline 106 (1981) in batch equilibrium experiments with a soil:solution ratio of 1:5.

A Freundlich isotherm was fitted to the data and resulted in a sorption coefficient K_d=2.90 cm^3 g^{-1} and a Freundlich exponent n=0.8.

I. MATERIALS AND METHODS

1. Test material

BAS code:	BAS 351 H
Common name:	Bentazone
Reg. No.:	51929
CAS-No.:	25057-89-0
Chemical name (IUPAC):	3-isopropyl-1H-2,1,3,-benzothiadiazin-4(3H)-one-2,2-dioxide
Chemical formula:	$C_{10}H_{12}N_2O_3S$
Molecular weight:	240.28

2. Test soils

A sandy loam soil from Mellby, Sweden, was used to determine Freundlich sorption of bentazone. The soil was sampled as undisturbed monolith lysimeter in the dry season of 1989.

According to the authors, the soil has a brownish black color, a weak coarse granular structure, and “is classified as a Fluventic Haplumbrept (U.S.D.A. System) or as a Haplic Phaeozem (F.A.O. system) with the following diagnostic characteristics: Mollic epipedon, Cambic horizon”.

The characteristics of the soil are summarized in Table 7.4/2.

Table 7.4/2 Characteristics of the experimental soil

Location	Mellby topsoil
Depth [cm]	0-23
Soil type (USDA specification)	Sandy loam
%Clay (< 2 µm) %Silt (2-50 µm) %Sand (50-2000 µm)	10.4 10.2 79.4
Organic matter [%]	5.9
pH(H_2O)	6.2
Porosity [m^3 m^{-3}] layer 0-10 cm Porosity [m^3 m^{-3}] layer 10-20 cm	0.47 0.42
Bulk density [g cm^{-3}] layer 0-10 cm Bulk density [g cm^{-3}] layer 10-20 cm	1.32 1.47

3. Experimental treatments

Freundlich sorption of bentazone was measured for topsoil samples (0 - 23 cm) in batch equilibrium experiments with a soil:solution ratio of 1:5. Sorption experiments were performed according to OECD guideline 106.

4. Sampling and analysis

Bentazone analysis in water samples was performed by hydrolyzing a 200 ml sample with 1 g KOH for 1 h at 100 °C to include esters and bound residues of bentazone. The sample was then acidified with about 5 mL concentrated H_3PO_4 and extracted twice with dichloromethane (50 ± 25 mL), followed by extractive alkylation with pentafluorobenzyl-bromide and evaporation to dryness of the dichloromethane phase. The remainder was re-dissolved in 1.5 mL hexane and analyzed by capillary column gas chromatography, with a detection limit of 0.1 µg/L.

5. Method validation

Analytical recovery was determined by fortification experiments over a concentration range of 0.1 to 5 µg/L.

II. RESULTS AND DISCUSSION

A. Method validation

Recoveries were between 80% and 120% in fortification experiments over the tested concentration range of 0.1 to 5 µg/L.

B. Findings

The results of the sorption experiment are presented in Table 7.4/3.

Table 7.4/3 Sorption of bentazone in Mellby topsoil

Sorption isotherm	K_d [$cm^3\ g^{-1}$]	n [-]	r^2 [-]
Linear	1.67	1.0	0.98
Freundlich	2.90	0.8	0.97

III. CONCLUSION

Sorption of bentazone was measured in a sandy loam soil with a pH of 6.2 and an organic matter content of 5.9 %. A Freundlich isotherm was fitted to the data and resulted in a sorption coefficient K_d=2.90 $cm^3\ g^{-1}$ and a Freundlich exponent of n=0.8. The corresponding $K_{f,oc}$ is 49.2 mL/g.

Report:	II A 7.4.1/2 Keller W. 1995(b) Adsorption study of Bentazon in a Vredepeel soil from the Netherlands BASF DocID 1995/10689
Guidelines:	OECD 106
Testing Laboratory and dates:	BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 01-Jul-1995 - 31-Aug-1995
GLP:	No

Executive Summary

In laboratory batch experiments the adsorption behaviour of radiolabelled bentazone was investigated on a Vredepeel soil from the Netherlands. The soils had a pH ($CaCl_2$) of 5.2 and an organic carbon content of 3.0 %. It was characterized as a loamy sand.

For the determination of the adsorption isotherm, five different concentrations from ca. 0.02 to 25 µg/mL of the test item in 0.01 M $CaCl_2$ solutions were used. The ratio of soil versus test solution was 2/1, and the equilibrium shaking time was 16 hours.

From the Freundlich adsorption isotherm, an adsorption constant K_f of 0.1933 mL/g and a Freundlich exponent 1/n of 0.9703 were determined. The corresponding $K_{f,oc}$ value was 6.4.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material	Bentazone BAS 351 H (Reg. No. 051929) [phenyl-U-^{14}C]-labelled: specific activity 5.21 MBq/mg (312660)
Lot/Batch #:	410-02, radiochemical purity 100.0%
CAS #:	25057-89-0
Molecular weight:	240.28 g/mol (unlabelled)

2. Soils

The study was conducted with a soil originating from the Netherlands from the location Vredepeel. The soil was taken from the top layer 0 - 30 cm. The soil characterization is given in Table 7.4/4:

Table 7.4/4 Characterisation of soil used to investigate the adsorption behaviour of bentazone

Soil designation Origin	Vredepeel, 0-30 cm layer Netherlands
Textural class	Loamy sand
Particle size [%] >200 µm: 20 - 200 µm: 6—20 µm: 2-6 µm: <2 µm:	 38 50 3 1 8
Organic carbon [%]	3.0
CEC [$cmol^+/kg$]	21.1
pH ($CaCl_2$)	5.2

The soils used were air-dried and sieved to a particle size < 2 mm. The residual water content of the soils was determined by further drying to constant weight at 105 °C.

B. STUDY DESIGN

1. Experimental conditions

The adsorption study was carried out at room temperature (20 - 25 °C).

To determine the incubation time for reaching equilibrium conditions, experiments were run with a soil / solution ratio of 2/1 (10 g/ 5 mL) for 1, 2, 4, 8, 16 and 24 hours on a mechanical shaker. The ^{14}C-bentazone concentration in the solution was 0.0225 µg/mL. After the corresponding shaking time, the soil-water suspension was centrifuged at 4000 rpm at 20 °C for 15 min. The aqueous phase was decanted and 1 mL aliquots were measured by liquid scintillation counting (LSC). The equilibrium test was carried out in duplicates.

Freundlich adsorption isotherm
Test solutions were prepared in the concentrations as follows by fortifying solution F with increasing amounts of cold bentazone:

Solution	Bentazone concentration [µg/ml]
A	25.0225
B	5.0225
C	1.0225
D	0.2225
E	0.0625
F	0.0225

To establish the Freundlich adsorption isotherm, 10 g aliquots of the soil were shaken with 5 mL of the ^{14}C-bentazone test solutions A - F, respectively, for 16 hours on a mechanical shaker. The samples were processed as described above. To show that no test substance adsorbed on the glass wall of the centrifuge tubes, ^{14}C-bentazone test solution F (0.0225 µg/mL ^{14}C-bentazone) without addition of soil was also shaken for 16 hours and analysed by LSC measurement.

Blank soil samples without test substance were also analysed to show that no radioactivity was in the used soil, which could interfere with the ^{14}C-measurements. The Freundlich adsorption isotherm was carried out in triplicates.

2. Description of analytical procedures

Radioactivity in the aqueous supernatants after centrifugation of the soil-water suspension was determined by LSC measurement.

II. RESULTS AND DISCUSSION

1. Adsorption equilibrium test

The equilibrium test, carried out with a shaking time period from 1 to 24 hours, revealed no formation of an exact plateau value. For practical reasons, a shaking time of 16 hours was chosen for the following Freundlich isotherm.

2. Freundlich Isotherm test

In Vredepeel soil a Freundlich adsorption constant K_f of 0.1933 mL/g and a Freundlich adsorption exponent 1/n of 0.9703 were determined for bentazone. The corresponding $K_{f,oc}$ value was 6.4 ml/g.

III. CONCLUSION

Using six test substance concentrations from ca. 0.02 - ca. 25 µg/ml, a Freundlich adsorption isotherm for bentazone was established. The equilibrium shaking time was 16 hours at room temperature. The soil / water ratio was 2 to 1.

For bentazone the value for the Freundlich adsorption constant K_f was 0.1933 mL/g, and the Freundlich adsorption exponent 1/n 0.9703. The corresponding $K_{f,oc}$ value was 6.4 ml/g.

Report:	II A 7.4.1/3 Seher A. 1999(b) Adsorption study of 51929 (BAS 351 H) on lysimeter soils BASF DocID 1999/10685
Guidelines:	OECD 106; EPA 163-1
Testing Laboratory and dates:	BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 01-Mar-1999 - 31-Mar-1999
GLP:	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

The adsorption behaviour of bentazone was determined on two lysimeter soils. For the determination of the adsorption isotherm, four different concentrations (nominal 5, 1, 0.2 and 0.04 µg/mL) of the test substance in 0.01 M $CaCl_2$ solution were prepared. Two lysimeter soils (both loamy sands) were selected for the experiments. The soil / water ratio was adjusted to 1/1. Equilibration time was checked in pre-test and was reached after 4 hours.

The following values of the Freundlich adsorption isotherm were measured for the two soils :

	Speyerer Wald	**Borstel**
Adsorption constant K_f [mL/g]	0.021	0.071
$K_{f,oc}$ [mL/g]	3.0	5.9
Adsorption exponent 1/n	0.849	0.980

I. MATERIAL AND METHODS

1. Test Material

Bentazone (Reg. No. 51929)
[phenyl-U-^{14}C]-labelled: specific activity 5.21 MBq/mg
Lot/Batch #: 410-05, radiochemical purity > 98%
CAS #: 25057-89-0
Molecular weight: 240.28 g/mol (unlabelled)

2. Soils

Two German Lysimeter soils were used in the experiments: Speyerer Wald and Borstel. The physicochemical soil parameters are listed in Table 7.4/5.

Table 7.4/5 Characterisation of soils used to investigate the adsorption behaviour of bentazone

Soil designation **Origin**	**Speyerer Wald** **Germany**	**Borstel** **Germany**
Textural class (German scheme, DIN 4220)	silty sand / loamy sand	sand/ loamy sand/ silty sand
Soil texture [%], (German scheme) Sand Silt Clay	 85 8 7	 85 10 5
Textural class (USDA scheme)	loamy sand	loamy sand
Soil texture [%], (USDA scheme) Sand Silt Clay	 85 7 7	 86 9 5
Organic carbon [%]	0.7	1.2
CEC [$cmol^+/kg$]	5	8
pH ($CaCl_2$) pH (water)	6.0 6.8	5.7 6.3

The soil samples were air-dried and sieved to particle size < 2 mm. The residual water content was determined by drying the soils at 105°C until to weight constant. It ranged between 0.54 and 0.84 % and was taken into account for all calculations.

B. STUDY DESIGN

1. Experimental conditions

Bentazone standard solution
Approximately 1.3 mg ^{14}C-bentazone and 3.7 mg non-labelled bentazone were weighed in a 1000 mL volumetric flask and dissolved in 1000 mL 0.01 M aqueous $CaCl_2$ solution. Aliquots of this solution A were checked by LSC measurements. This solution A showed a test concentration of nominal 5 µg/ml with a specific activity of 3.8 x 10^5 dpm/mL. Solutions B, C and D (nominal concentrations 1, 0.2 and 0.04 µg/mL, respectively) were prepared by subsequent dilution of solution A.

Adsorption equilibrium test
To determine the time until equilibrium conditions will be reached, samples of 20 g soil and 20 mL solution A were filled in 150 mL centrifuge glass tubes. The tubes were covered with parafilm to avoid evaporation of water. The tubes were gently shaken on a mechanical shaker for 1, 2, 4, 8, 16 and 24 hours in the dark. The soil/water suspension was then centrifuged and the supernatant was decanted from the soil residue. An aliquot of the supernatant was then radio-assayed. A control was carried out with 20 g soil and 20 mL 0.01 M aqueous $CaCl_2$ solution without test substance. The results showed that the equilibrium time was reached after 4 hours.

To check if the test substance was adsorbed to centrifuge glass tube, a 20 mL aliquot of the solution A was also carried out without soil by shaking 24 hours along with the equilibrium test.

Adsorption isotherm determination
The four test substance concentrations of nominal 5, 1, 0.2 and 0.04 µg/mL (solutions A, B, C, D) were prepared and the adsorption isotherm determination test was performed with each soil as described for the adsorption equilibrium test. After centrifugation and decantation, the volume of the supernatant was measured by weighing and an aliquot analysed by LSC. Control measurements for the solutions B, C, D were also performed without soil as described under "adsorption equilibrium test".

2. Description of analytical procedures

Radioactivity in the aqueous supernatants was determined by liquid scintillation counting (LSC). Radio-HPLC was used to show the purity of the test substance. The solution A was analysed before and at the end of the study.

II. RESULTS AND DISCUSSION

1. Preliminary test

The measurements of the adsorption equilibrium test, carried out at various shaking times from 1 to 24 hours, revealed that the equilibrium plateau was reached after 4 hours. The equilibrium conditions were considered reached when results of two following measurements were similar within a 10 % deviation range.

2. Freundlich Isotherm test

The Freundlich adsorption coefficients K_f and the corresponding Freundlich adsorption exponent 1/n as well as the corresponding $K_{f,oc}$ values are given in Table 7.4/6. The Freundlich adsorption exponent 1/n indicated a non-linearity of the adsorption with the concentration.

Table 7.4/6 Results from the adsorption experiments with bentazone

Soil	Soil Type (USDA)	Org. C [%]	pH ($CaCl_2$)	K_f [mL/g]	1/n	$K_{f,oc}$ [mL/g]
Speyerer Wald (98/1287)	Loamy sand	0.7	6.0	0.021	0.849	3.0
Borstel (99/1332)	Loamy sand	1.2	5.7	0.071	0.980	5.9

3. Mass balance

A mass balance for the two soils was not established. However the stability of the test item was proven by HPLC in the aqueous solution after adsorption for the test solution A (initial concentration 5 µg/mL) in both soils. The purity of the test item was 99.58% in soil Speyerer Wald and 99.61% in soil Borstel indicating stability of the test item in the solution.

III. CONCLUSION

The adsorption and desorption behaviour of the test item bentazone was determined on two Lysimeter soils (Speyerer Wald and Borstel) with pH values of 6.0 and 5.7 and organic carbon contents of 0.7% to 1.2%.

Bentazone is adsorbed to the soils with measured K_f values of 0.021 and 0.071 mL/g and corresponding $K_{f,oc}$-values from 3.0 and 5.9 mL/g. The test substance was stable (> 99 %) during the adsorption procedure.

Scientific publications

The following four publications all refer to the same data set based on experiments described in a PHD thesis of *Boivin* 2003 (written in French). Three scientific papers in English prepared from this thesis are included in this dossier. Two are dealing with the adsorption values obtained in 13 soils, the third one dealing with aged sorption effects in selected three out of the 13 soils.

A fifth publication (*Larsbo* et al., 2009) is summarized where the adsorption behaviour of bentazone was described in three Swedish soils under different tillage systems.

Report:	II A 7.4.1/4 Boivin A. 2003(b) Disponibilité spatio-temporelle et transfert des pesticides dans le sol BASF DocID 2003/1032519
Guidelines:	OECD 106
Testing Laboratory and dates:	24-Oct-2003
GLP:	No, not subject to GLP regulations (PHD thesis)

Report:	II A 7.4.1/5 Boivin A. et al. 2005(b) A comparison of five pesticides adsorption and desorption processes in thirteen contrasting field soils BASF DocID 2005/1041040
Guidelines:	OECD 106
Testing Laboratory and dates:	27-Apr-2005
GLP:	No, not subject to GLP regulations (scientific publication)

Report:	II A 7.4.1/6 Boivin A. et al. 2005(c) Bentazone adsorption and desorption on agricultural soils BASF DocID 2005/1041502
Guidelines:	OECD 106
Testing Laboratory and dates:	03-Jan-2005
GLP:	No, not subject to GLP regulations (scientific publication)

Executive Summary

In laboratory batch experiments the adsorption / desorption behaviour of five pesticides including bentazone was investigated on thirteen European agricultural top soils according to the current version of the OECD guidance 106 (2000). The soils covered a range of pH (H_2O) from 5.3 to 8.2, a range of organic matter content from 1.08 to 6.03% and different textural classes. In the following the data obtained for bentazone are reviewed.

For the determination of the adsorption isotherm, four different concentrations (nominal 0.25, 1, 5 and 10 mg/l) of the test item in 0.01 M $CaCl_2$ solutions were used. Non-radiolabelled and ^{14}C-labelled bentazone was mixed to obtain the respective concentrations. The ratio of soil versus test solution was 1/5, and the measurements were performed at the adsorption equilibrium time of 16 hours for the thirteen soils.

Desorption was also investigated starting at a concentration of 1 mg/L. Supernatants were replaced by aqueous $CaCl_2$ solution and tubes were shaken for 16 h at 20±2 °C. The desorption process was repeated until the supernatant radioactivity became less than three times the liquid scintillation analyser background noise.

Freundlich adsorption coefficients (K_f) between 1.2 and 1.9 mL/g were calculated. Freundlich exponents (1/n) of 0.99 or 1.0 were determined, indicating linearity of the sorption isotherms. $K_{f,oc}$ – values range from 36 to 158 mL/g.

In the desorption experiments, a large part of bentazone was released without any significant differences between the thirteen soils (96.9± 4.3%).

I. MATERIAL AND METHODS

1. Test Material [U-^{14}C-phenyl]bentazone
specific activity 6.69 MBq/mg
radiochemical purity > 99%
Molecular weight: 240.28 g/mol (unlabelled)

A non-radio-labelled solution was prepared separately with aqueous calcium chloride solution (0.01 M) and then mixed with ^{14}C-labeled methanol solutions to achieve concentrations of 0.25, 1, 5 and 10 mg/L. Methanol represented less than 0.01% in the final solutions (cited from Boivin *et al.* 2005).

2. Soils

Thirteen cultivated soils were sampled in the surface layers (0 - 15 cm). They were selected on the basis of their texture, organic matter contents and pH values. Twelve soils came from Lorraine area (France) and one from Brittany (Experimental station of Kerlavic; France). Soil types were classified into cambisols, calcisols and regosols according to the FAO classification [cited from Boivin *et al.* 2005].
The physico-chemical characterization of the soils is provided in Table 7.4/7.

Table 7.4/7 Characterization of soils used to investigate the adsorption behaviour of bentazone

Soil type	Clay [%]	Loam [%]	Sand [%]	Organic matter content [%]	pH_{H2O} [-]	Organic carbon-nitrogen ratio [-]	Cation exchange capacity [cmol/kg]
Dystic Cambisol	17.7	45.2	37.1	6.03	5.3	10.50	13.5
Stagnic Cambisol	53.6	39.7	6.7	5.81	6.7	9	31.5
Calcaric Regosol 2	38.1	34.2	27.7	5.59	8.2	8.83	24
Fluvic Gleyic Cambisol	51.6	42.9	5.5	4.80	6.2	8.66	25.4
Eutric Cambisol 2	10.4	19	70.6	3.66	6.2	10.47	7.9
Calcaric Regosol 1	33.4	25	41.6	3.63	7.9	8.66	14.7
Cambic Calcisol	45.5	44.3	10.2	3.64	8.1	10.59	16.9
Vertic Stagnic Cambisol	41.1	48.6	10.3	3.32	6.9	9.90	18.7
Cambic Stagnic Vertic Calcisol	50.9	34.8	14.3	2.77	8	7.92	21.2
Stagnic Luvisol	30.9	50	19.1	2.62	5.9	9.13	5.5
Fluvic Stagnic Cambisol	11.4	22.5	66.1	1.50	5.8	9.6	14.8
Eutric Cambisol 1	12.7	13.8	73.5	1.43	6.4	10.13	4.6
Fluvic Cambisol	20.1	55.4	24.5	1.08	5.5	9.29	9.6

Soil samples were air-dried, sieved to 2-mm, stored in the dark at room temperature (20 ± 2 °C) and sheltered from humidity.

B. STUDY DESIGN

1. Experimental conditions

Sorption isotherms were carried out using the standard batch equilibration method according to the OECD guideline 106 (2000).

In the first experiment, aqueous calcium chloride solutions (0.01 M, 10 mL) were added to three replicates of 2 g of air-dried, sieved soil, in 25 mL Corex™ glass centrifuge tubes, in order to bring the samples to water saturation. Tubes were shaken with a rotary agitator (60 rpm) for 1 h at 20 ± 2 °C in the dark and then centrifuged at 5000 g for 25 min. Next, supernatants were taken (8 mL), replaced by labelled solutions, after which the tubes were shaken again (same conditions as before). According to a previous kinetic study (Boivin, A., et al. 2004, see study 7.4.1/7 described below), equilibrium was assumed to be reached within the 16 h equilibration period and that degradation can be neglected during the batch equilibrium period.

Desorption experiments were conducted immediately after the sorption experiments to prevent further degradation, starting at a concentration of 1 mg/L, by successive dilution. First, supernatants were replaced with an aqueous calcium chloride solution (0.01 M, 8 mL), after which the tubes were shaken at 20 ± 2 °C for 16 h. During the preparation of the different repetitions, the tubes were stored in a fridge (5 °C) to avoid biodegradation. The suspensions were subsequently centrifuged and the radioactivity of the supernatant was measured (similarly as for the sorption experiments). The desorption process was repeated using the above procedure until the supernatant radioactivity became three times less than the liquid scintillation analyser background noise (1.7 Bq) [cited from Boivin *et al.* 2005].

2. Description of analytical procedures

Radioactivity in the aqueous supernatants was determined by liquid scintillation counting (LSC). Several tubes without soil were also shaken to serve as a control; they showed no loss of ^{14}C. Thus, differences between the initial and equilibrium concentrations were assumed to be due to sorption onto soil.

II. RESULTS AND DISCUSSION

1. Freundlich Isotherm test

The Freundlich adsorption coefficients K_f ranged from 1.2 to 1.9 mL/g for the thirteen soils (Table 7.4 /8). Ranging from 1/n = 0.99 to 1, the Freundlich adsorption exponent indicated linearity of the adsorption with the concentration. On average, 22 ± 2.3 % of the total amount were adsorbed.

Table 7.4 /8 Results from the adsorption experiments with bentazone

Soil type	pH_{H2O} [-]	Adsorption [%]	K_f [mL/g]	$K_{f,oc}$* [mL/g]	1/n [-]
Dystic Cambisol	5.3	28 ± 1	1.9 ± 0.2	55	0.99
Stagnic Cambisol	6.7	22 ± 1	1.4 ± 0.1	41	1
Calcaric Regosol 2	8.2	20 ± 2	1.2 ± 0.1	36	0.99
Fluvic Gleyic Cambisol	6.2	23 ± 1	1.5 ± 0.1	54	1
Eutric Cambisol 2	6.2	21 ± 1	1.3 ± 0.2	60	1
Calcaric Regosol 1	7.9	20 ± 2	1.2 ± 0.1	63	1
Cambic Calcisol	8.1	20 ± 4	1.3 ± 0.1	55	1
Vertic Stagnic Cambisol	6.9	20 ± 1	1.2 ± 0.1	63	1
Cambic Stagnic Vertic Calcisol	8	20 ± 1	1.3 ± 0.1	79	1
Stagnic Luvisol	5.9	22 ± 1	1.4 ± 0.1	93	1
Fluvic Stagnic Cambisol	5.8	22 ± 1	1.4 ± 0.1	137	1
Eutric Cambisol 1	6.4	20 ± 1	1.2 ± 0.1	158	0.99
Fluvic Cambisol	5.5	23 ± 2	1.5 ± 0.1	144	0.99

* Taken from II A 7.4.1/4 – DocID 2003/1032519 (page 64)

Principal component analysis corroborated this finding, indicating that K_f values and soil pH were inversely related, while other soil components showed no or little effect. The two main principal component axes could explain 94.57% of the total variability.

Furthermore, multiple linear regression was used to relate K_f values with organic matter content and soil pH.

2. Desorption behaviour

A large part of bentazone could be desorbed from the soil without any significant differences between the thirteen soils (96.9% ± 4.1%). No significant multiple correlation coefficient could be found for the relationship between desorption and the soil properties organic matter and pH.

III. CONCLUSION

The adsorption behaviour of bentazone was determined on thirteen European agricultural top soils, which covered a range of pH from 5.3 to 8.2 and a range of organic matter content from 1.08 to 6.03% and different textural classes.

Freundlich adsorption coefficients K_f ranged from 1.2 to 1.9 mL/g for the thirteen soils. Freundlich exponents were between 0.99 and 1.0 indicating linearity of the adsorption isotherm. $K_{f,oc}$ –values range from 36 to 158 mL/g. A correlation was found between soil pH and K_f value, with slightly higher adsorption at lower soil pH. Using multiple linear regression a model was found where bentazone K_f values were predicted using soil organic matter content and pH.

Report:	II A 7.4.1/7 Boivin A. et al. 2004(b) Time effect on Bentazone sorption and degradation in soil BASF DocID 2004/1015218
Guidelines:	None
Testing Laboratory and dates:	19-Apr-2004
GLP:	No, not subject to GLP regulations (scientific publication)

Executive Summary

The sorption and degradation behaviour of bentazone was investigated in three French soils over a time range of 160 days. The soils were characterized as a clay soil, a loamy soil and a sandy soil. The soils were passed through a 2 mm sieve before use. For treatment, a mixture of radiolabeled (phenyl-U-^{14}C) and non-radiolabeled bentazone was used. The test item was applied in separate batch applications at a nominal rate of 0.5 mg bentazone per sample (50 g soil) which, according to the authors, corresponds to a field application rate of 1200 g bentazone per hectare.

Soil batches were incubated in the dark under aerobic conditions at a soil moisture of 80% field capacity and a temperature of 20°C. Each soil sample was placed in a glass dish in an individual airtight jar (1.5L), containing flasks with aqueous sodium hydroxide (10 ml 0.5 M) for trapping evolving $^{14}CO_2$, and with distilled water for keeping sufficient humidity in the surrounding atmosphere to reduce water losses from the soil. Samples were taken at 0, 7, 15, 30, 60 and 160 days after treatment (DAT).

Soil samples were extracted several times with 0.01 M $CaCl_2$ solution for 16 h, respectively, until the supernatant radioactivity became less than three times the background radioactivity. Subsequently, the soil samples were extracted with methanol until the supernatant radioactivity became less than three times the background radioactivity. The individual extracts were analysed by liquid scintillation counting (LSC). The remaining soil after extraction was combusted in order to determine the amount of non-extractable soil bound residues.

To establish extraction kinetics for the extraction with 0.01 M $CaCl_2$ solution, subsamples of the extraction supernatant were taken after 1, 2, 5, 10, 20, 30, 60, 180, 420 and 1200 min of shaking. Approximately 90% of applied bentazone was released in less than 1 h and a quasi-equilibrium state was reached after 2 h.

At the beginning of the investigations, the amount of $CaCl_2$-extractable bentazone was high (92 – 97% of applied radioactivity at day 0) but decreased over time to 11-51% of applied radioactivity after 160 days of incubation. The number of extractions necessary to release the water-extractable portion of bentazone increased at increasing incubation time.

No information was given on the amount of bentazone in the $CaCl_2$ and the following methanol extracts. Therefore, a clear time dependent sorption of bentazone in soil could not be calculated. The reduced extractability of bentazone with $CaCl_2$ solution over time described in this publication is thus due to degradation processes which led to tight binding and incorporation into the organic soil matrix.

I. MATERIALS AND METHODS

1. Test Material

BAS code:	Bentazone
Chemical name (IUPAC):	3-isopropyl-1H-2,1,3,-benzothiadiazin-4(3H)-one-2,2-dioxide
Molecular weight:	240.3 g/mol (unlabelled)
Position of radiolabel:	U-phenyl-^{14} C
Specific radioactivity:	6.59 MBq/mg
Radiochemical purity:	>99%
Origin:	Isotopchim, France

2. Soil

Three agricultural soils from Lorraine (surface layers, 0 – 15 cm) were selected for the laboratory experiments: a vertic stagnic cambisol (clay soil), a stagnic luvisol (loamy soil) and a fluvic stagnic cambisol (sandy soil). The soils were air-dried and sieved to 2 mm.

A summary of the soil characteristics is given in Table 7.4/9.

Table 7.4/9 Properties of soils used to investigate sorption and degradation of bentazone under aerobic conditions

	Clay (%)	Loam (%)	Sand (%)	Organic matter content (%)	pH_{H2O}	Linked water [a] (cm^3 cm^{-3})	Mobile water [b] (cm^3 cm^{-3})	Organic carbon/nitrogen ratio	Cation exchange capacity (cmol kg^{-1})
Vertic stagnic cambisol (clay soil)	41	49	10	3.3	7	0.39	0.13	9.9	18.7
Stagnic luvisol (loamy soil)	31	50	19	2.5	5.9	0.33	0.10	9.1	14.8
Fluvic stagnic cambisol (sandy soil)	11	23	66	1.5	5.8	0.27	0.21	9.6	5.5

a Soil water content estimated at -20 mm H_2O.

b Soil water content estimated at saturation (θ_S).

3. Study Design

Experimental conditions

Soil aggregates (50 g) were placed in glass dishes (6 cm diameter). Water was added to each soil sample to 80% of field capacity. Each soil sample was then treated on its surface with a mix of bentazone and ^{14}C-bentazone (59.5 kBq) in aqueous solution. The aqueous solution was applied to the whole soil surface by simulating micro-drops with a micro-syringe. The amount of added herbicide represented 0.5 mg per sample and was (according to the authors) equivalent to a 1200 g/ha field treatment. Three repetitions were done per soil and blanks were made up with 50 g of soil and the same volume of water. Each soil sample was then placed in an individual airtight jar (1.5 litre), containing flasks of aqueous sodium hydroxide (0.5 M; 10 mL) and distilled water. The sodium hydroxide trapped CO_2 evolved from the soil and ^{14}C-CO_2 from bentazone ^{14}C-ring degradation, and the distilled water flask reduced water losses from the soil by maintaining constant humidity in the system. The jars were stored in the dark at 20 (±1) °C. (cited from Boivin *et al.* 2004)

Sampling

Sampling times were 0, 7, 15, 30, 60 and 160 days after treatment (DAT).

Analytical procedures

Investigation of extractability of aged residues

Soil samples were rotary shaken at 20 (±1) °C with aqueous calcium chloride (0.01 M; 150 mL) in PTFE centrifuge flasks. For each time studied, the release kinetics of bentazone into water were determined by measuring the radioactivity present in the supernatant solution after shaking times of 1, 2, 5, 10, 20, 30, 60, 180, 420 and 1200 min. A sample (1 mL) was taken at each time interval, centrifuged at 5000 g for 20 min, and the radioactivity in the supernatant measured by liquid scintillation counting. At the end of the kinetic study, the soil/water mixture was centrifuged at 5000 g for 20 min, the supernatant replaced with fresh aqueous calcium chloride (0.01 M; 150 mL) and shaken as before for 16 h. This operation was repeated until the supernatant radioactivity became less than three times the background noise of the scintillator analyser (1.7 Bq). The bentazone release with $CaCl_2$ was found to follow a logarithmic time function. Approximately 90% of applied bentazone was extracted in less than 1 h and a quasi-equilibrium state was reached within 2 h.

To completely extract any remaining residues, the soil was then repeatedly extracted with methanol (100 mL) using the same procedure as for the $CaCl_2$ solution.

In addition to the experiments with $CaCl_2$ extraction followed by methanol extraction, a series of soils were extracted only with methanol and the methanol extracts then analysed by HPLC to determine potential extractable metabolites. However, an identification was not performed.

Non-extractable residues in soil

After exhaustive extraction of the residues with water and methanol, the soil pellets were air-dried and homogenized. Determination of non-extractable ^{14}C- residues was performed by combustion of soil samples (300mg aliquots). The ^{14}C-CO_2 evolved from the samples was trapped and the radioactivity was determined by liquid scintillation counting.

Data processing
Descriptive statistical analysis (variance analysis, principal component analysis) of the data and multiple linear regressions were performed with Statistica®.

II. RESULTS AND DISCUSSION

A. MINERALIZATION

Mineralization of bentazone was low (see Table 7.4/10) with levels between 2.7 and 14% of applied amount reached at the end of incubation (after 160 days). The authors discussed that mineralization seems to be correlated with the amount of organic matter (P = 0.05). The sandy soil with the lowest organic matter content (1.5%) showed the lowest rate of mineralization. Conversely, in clay soil with the highest organic matter content (3.3%) the highest rate of mineralization was found. Influence of pH was interpreted to be rather low, however, the pH of the tested soils did no cover a large range (pH 5.8 - 7).

Table 7.4/10 Mineralized amounts of bentazone [% total applied]

	Incubation time [days]					
	0	7	14	30	60	160
Clay soil	0.1	0.3	0.5	3.5	6.1	14
Loamy soil	0.1	0.2	0.35	2	4.5	11
Sandy soil	0.1	0.11	0.14	0.5	0.9	2.7

B. EXTRACTABILITY OF AGED RESIDUES

The amount of residues extractable with 0.01 M $CaCl_2$ and methanol was clearly influenced by the incubation time and the type of soil as indicated by variance analysis of desorption (P< 0.05).

Directly after application between 92 and 97% of applied radioactivity was extracted with 0.01 M $CaCl_2$ solution, whereas at the end of incubation between 11 and 51% of applied radioactivity were found (see Table 7.4/11). Furthermore the number of extractions necessary to release the water-extractable portion of bentazone increased at increasing incubation time.

The residues were easily extractable from the sandy soil, whereas smaller amounts were desorbed from the clay and the loamy soil. The authors explained this with an influence of soil structure on the sorption/desorption behaviour of bentazone residues, with a higher surface area and a higher number of micropores in the clay and loamy soil compared to sandy soil.

Lower amounts of bentazone residues were desorbed with the subsequent methanol extraction. The levels extracted with methanol reached a maximum after seven days (8 to 19% of applied radioactivity) and lowered again towards the end of incubation.

Conversely, both incubation time and soil type also influenced the amount of bound residues. In clay and loamy soil the amounts reached a maximum of about 65% of applied after 60 days of incubation (also found after 160 days). In the sandy soil lower amounts were found with a maximum of about 30% of applied after 160 days.

Table 7.4/11 Bentazone residues extracted with aqueous calcium chloride (0.01 M) followed by methanol over the range of times [% total applied]*

		Incubation time [days]					
		0	**7**	**14**	**30**	**60**	**160**
Clay soil	$CaCl_2$	94 (±2)	83 (±2)	75 (±2)	44 (±1)	18 (±1)	11(±1)
	Methanol	3 (±0.5)	8 (+2)	3 (±2)	2 (+2)	2 (±2)	1 (±1)
Loamy sand	$CaCl_2$	92 (±2)	81 (±2)	75 (+2)	51 (±2)	25 (+2)	15 (±1)
	Methanol	6 (±1)	10 (±1)	5 (±1)	4 (±1)	3 (±1)	2 (±1)
Sandy soil	$CaCl_2$	97 (±1)	77 (±2)	84 (±2)	79 (±2)	65 (±2)	51 (±2)
	Methanol	0.1 (±0.1)	19 (±2)	11 (±2)	7 (±2)	5 (±2)	3 (±1)

* Standard deviations are given in parentheses

No information was given on the amount of bentazone in the $CaCl_2$ and the following methanol extracts. Therefore, a clear time dependent sorption of bentazone in soil could not be calculated.

However, for the 7 and 14 day sampling times, the reduced extractability of bentazone with $CaCl_2$ solution and the increase of radioactivity in the methanol extracts indicates an increase of sorption and a start of movement into the micropores so that bentazone became less and less available to water desorption but still remained available to methanol extraction. This is also supported by the higher numbers of aqueous extraction cycles needed to reach < 3-fold background level in the LSC at later samplings (n=7) compared to the 0 day samplings (n=2). The bentazone fraction obviously became more and more difficult to reach.

At later samplings, degradation processes prevail and the extractability of radioactivity decreases in the $CaCl_2$ solution as well as in methanol.

C. CHARACTERIZATION OF METHANOL EXTRACTABLE RESIDUES

The HPLC analysis of methanol extracts of the soils not previously extracted by $CaCl_2$ revealed the presence of bentazone and also two metabolites at later stages of incubation. Metabolite identification was not performed. The authors mentioned 6- and 8-OH-bentazone as possible degradation products in soil, however, this is considered rather unlikely. These metabolites are not expected to occur in soil in measurable amounts because they will be immediately incorporated into the humic matrix by oxidative coupling. It is much more likely that N-methyl-bentazone was formed. The nature of the second metabolite described in this paper remains unknown. However, none of the metabolites could have exceeded amounts of 5 - 10% of the applied radioactivity. Clear values could unfortunately not be calculated from presented results since extractability data from pure methanol extraction were not provided.

III. CONCLUSION

The degradation and sorption behaviour of ^{14}C-bentazone was investigated in three different soils. The $CaCl_2$ extractability of radioactivity decreased from 92 - 94% at day 0 to 11 - 51% at day 160, whereas levels of the subsequent methanol extracts increased considerably within the first 14 days (max. 8 to 19% after 7 days). Apparently, bentazone residues reach a stage of sorption were they are less accessible to water but still extractable with methanol. At later samplings, degradation processes prevail and the extractability of radioactivity decreases in the $CaCl_2$ solution as well as in methanol. The formation of bound residues could be linked to the soil organic matter content.

Report:	II A 7.4.1/8 Larsbo M. et al. 2009(a) Herbicide sorption, degradation, and leaching in three Swedish soils under long-term conventional and reduced tillage BASF DocID 2009/1127645
Guidelines:	OECD 106
Testing Laboratory and dates:	19-Aug-2009
GLP:	No, not subject to GLP regulations (scientific publication)

Executive Summary

In laboratory experiments, the adsorption behaviour of bentazone was determined on three Swedish soils under different tillage systems according with the OECD guideline 106 (2000). The soils covered a range of pH (in water) from 5.3 to 6.7, a range of organic carbon content from 1.8 % to 3.0 % and different textural classes (silty clay, loam and sandy loam).

For the determination of the adsorption isotherm, five different concentrations in the range of 0.075-10 µg/g dry weight of the test item in 0.01 M $CaCl_2$ solutions were used.

The resulting Freundlich adsorption coefficient K_f covered a range from 0.148 to 0.567 mL/g, with the Freundlich adsorption exponent ranging from 1/n = 0.767 to 1.11. Freundlich adsorption coefficients were largest (though not always significantly) for $RT_{0–5\ cm}$ for all sites. Since the differences in n_f-values were in most cases small, this indicates stronger sorption for $RT_{0–5\ cm}$ at all concentrations.

I. MATERIAL AND METHODS

1. Test Material

Unlabelled bentazone (3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide; 97% purity) was purchased from Dr. Ehrenstorfer GmbH, Augsburg, Germany. ^{14}C-labeled bentazone (3-(1-methylethyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide-[phenyl-U-14C]; 5.211 MBq/mg; 100% chemical and radiochemical purity) was provided by BASF, Limburgerhof, Germany. Potassium chloride (KCl; purity 99.5%) and potassium bromide (KBr; purity 99.5%) were purchased from Merck KGaA, Darmstadt, Germany.

2. Soils

The study was conducted with three different soils originating from long-term tillage experimental plots in Sweden. The long-term tillage experiments were established in 1974 at Ultuna and Lönnstorp and in 1997 at Säby. For all sites conventional tillage (CT) means shallow cultivation (stubble cultivation by discing or chiseling) followed by moldboard ploughing. Reduced tillage (RT) means only shallow cultivation. The depth of influence for CT and RT is about 25 and 10 cm, respectively. All samplings were made in June 2006 for Ultuna and Säby and in June 2007 for Lönnstorp (i.e. about 4–6 weeks after sowing).

The physico-chemical characterization of the soils is provided in Table 7.4/12.

Table 7.4/12 Characterization of soils used to investigate the adsorption behaviour of bentazone

Soil designation	Ultuna 59°49'N; 17°39'E			Säby 59°50'N; 17°42'E			Lönnstorp 55°40'N; 13°6'E		
Textural class	Silty clay			loam			Sandy loam		
Depth [cm]	0-20	0-5	10-20 **	0-20	0-5	10-20 **	0-20	0-5	10-20 **
Tillage *	CT	RT	RT	CT	RT	RT	CT	RT	RT
Clay (<2 µm)	45.8	44.0	51.7	21.0	21.3	19.5	16.0	15.6	15.5
Silt (2–60 µm)	39.2	42.4	40.9	47.3	50.8	48.2	27.2	27.8	28.0
Sand (60-200 µm)	15.0	13.6	7.4	31.7	27.9	32.3	56.8	56.6	56.5
pH (H_2O) **	5.34 ±0.42	5.63 ±0.18	5.67 ±0.036	5.56 ±0.19	5.71 ±0.11	5.71 ±0.15	6.73 ±0.53	6.22 ±0.07	6.37 ±0.035
Total organic carbon [%] **	1.97 ±0.45	2.57 ±0.18	1.94 ±0.45	2.75 ±0.1	3.04 ±0.007	2.45 ±0.14	1.8 ±0.23	2.14 ±0.034	1.79 ±0.14

* CT = conventional tillage, RT = reduced tillage
** The total organic carbon content and pH were measured in samples from 12–17 cm depth. Results are given ± standard deviation (n=4 for Ultuna and Säby; n=3 for Lönnstorp)

B. STUDY DESIGN

1. Experimental conditions

Adsorption data were obtained for five different concentrations for two replicate samples between 0–5 and 10–20 cm depths for RT ($RT_{0–5\ cm}$, $RT_{10–20\ cm}$) and the 0–20 cm depth for CT for all sites. Two gram dry weight of soil was shaken for pre-equilibration with 1.9 ml of 0.01 M $CaCl_2$ for 12 h at 20 °C in 10 mL glass tubes. Thereafter, the soil slurry was spiked with 0.5 mL of a mixture of labelled (7000–11000 dpm) and unlabelled bentazone in 0.01 M $CaCl_2$ to give five initial concentrations in the range of 0.075–10 µg/g dry weight for bentazone. After shaking for 24 hours, the tubes were centrifuged for 30 min at 3000 rpm, after which the radioactivity was measured in 1 mL of the supernatant by liquid scintillation counting. Tubes without soil and ^{14}C-labeled substances were included for subtraction of background radiation, and tubes without soil were used to give the initial amount of ^{14}C activity added.

2. Description of analytical procedures

Bentazone was extracted from the soil samples with 20 mL of methanol by shaking for 1 h at 200 rpm. The samples were centrifuged for 20 min at 1500 rpm, and 1 mL aliquots of the supernatants were analysed using high performance liquid chromatography (HPLC).

Model fits for the adsorption models were carried out using the non-linear regression tool (PROC NLIN) in the SAS software version 9.1 (SAS Institute, 2002).

II. RESULTS AND DISCUSSION

A. METHOD VALIDATION

The recoveries for bentazone were 114–119% for all the studied soils. The limit of quantification (LOQ) was 0.07 mg/g dry weight. The limit of detection (LOD) was determined to be one third of this value.

No significant adsorption of bentazone occurred on the glass tubes.

B. FREUNDLICH ADSORPTION ISOTHERM DETERMINATION

The Freundlich isotherm was fitted to the measured data using linear regression on log-transformed data. Freundlich adsorption coefficients K_f covered a range from 0.148 to 0.567 mL/g, with the Freundlich adsorption exponent ranging from 1/n = 0.767 to 1.11. The results are summarized in Table 7.4/13.

Freundlich adsorption coefficients were largest (though not always significantly) for $RT_{0–5\ cm}$ for all sites. Since the differences in n_f-values were in most cases small, this indicates stronger sorption for $RT_{0–5\ cm}$ at all concentrations.

Table 7.4/13 Results from the adsorption experiments with bentazone in Swedish soils under different tillage systems

Soil			K_f [mL/g]	1/n [-]
Ultuna	CT*	0–20 cm	0.479	0.846
Ultuna	RT*	0–5 cm	0.567	0.777
Ultuna	RT	10–20 cm	0.557	0.767
Säby	CT	0–20 cm	0.371	0.847
Säby	RT	0–5 cm	0.396	0.864
Säby	RT	10–20 cm	0.339	0.857
Lönnstorp	CT	0–20 cm	0.323	1.11
Lönnstorp	RT	0–5 c m	0.440	0.942
Lönnstorp	RT	10–20 cm	0.148	0.880

* CT = conventional tillage, RT = reduced tillage

III. CONCLUSION

The adsorption behaviour of bentazone was determined on three Swedish soils under different tillage systems. The soils covered a range of pH (in water) from 5.3 to 6.7, a range of organic carbon content from 1.8 % to 3.0 % and different textural classes (silty clay, loam and sandy loam). The Freundlich adsorption coefficient K_f covered a range from 0.148 to 0.567 mL/g, with the Freundlich adsorption exponent ranging from 1/n = 0.767 to 1.11.

Overall summary on adsorption of bentazone on soils

In total seven different sources (studies) including 28 different soils were considered for the selection of appropriate sorption parameters for further purpose such as exposure modelling.

Two studies were already peer reviewed during the first process of Annex I inclusion [Redeker, 1978; Keller 1986] and the agreed endpoints were reported in the EU review report of bentazone as shown in Table 7.4/1.

Apart from the parameters derived from those studies, sorption values of three additional studies not previously peer-reviewed in the European registration procedure were considered valid and were additionally included in the parameter values for PEC calculation (Bergström et al., 1994; Keller, 1995; Seher, 1999). These values have been used for product registration in Europe since Annex I listing of bentazone (2000) as shown in Table 7.4/1. Modelling endpoints based on values of Table 7.4/1 (EU Endpoints from the review report including three additional studies) have been accepted from member states as relevant parameters. All additional studies (Bergström et al., 1994; Keller, 1995; Seher, 1999) were carried out with ^{14}C-labeled bentazone according to the OECD 106 guideline for testing the adsorption/desorption behaviour of chemicals. The studies are considered reliable to be used for parameter selection and OECD summaries of the studies are included in Section II A 7.4.1/1 to II A 7.4.1/3 of this dossier supplement.

As a result of literature search two additional sorption studies were found which were also considered relevant [Boivin, 2003, Boivin et al., 2005a+b; Larsbo et al., 2009]. Both studies were carried out with ^{14}C-labeled bentazone according to OECD 106. Potential losses during the adsorption process by adsorption to the walls of the glass vials as well as degradation during the shaking procedure was tested and found to be negligible. The studies are considered reliable to be used for parameter selection and OECD summaries of the studies are included in Section II A 7.4.1/4 to II A 7.4.1/6 and Section II A 7.4.1/8 of this dossier supplement.

Due to the low sorption of bentazone to soils, the P-criterion which is one of the quality criteria according to OECD 106 guideline (the product of K_f-value and the soil/water-ratio must not below 0.3) was not passed for all soils. Therefore, all $K_{f,oc}$-value values, regardless if resulting from EU peer-reviewed studies or from additional studies were checked for the P-criterion. All soils not passing the P-criterion were subjected to a correction procedure. The method for correction is described in (Boesten et al., 2009, http://www.yorkpesticides2009.com/poster/A11_Boesten.pdf) and accounts for possible losses of the substance due to degradation or adsorption to the walls of the test vials. The correction procedure considers differences between actual recovery and 100% as material not adsorbed to soil, and the $K_{f,oc}$-value is accordingly corrected. This means, the lower the recovery, the greater the correction effect on the $K_{f,oc}$-value.

An overview of all sorption values is shown in Table 7.4/14, listing the original $K_{f,oc}$-values as well as the corrected values of those soils, which did not pass the P-criterion. In Larsbo et al. (2009), three different treatments of the same soils were tested, all resulting in similar sorption parameters. Therefore, the values of the same soils were averaged and only one $K_{f,oc}$ and 1/n-value per soil was used in order to avoid multiple weighting of single soils.

The arithmetic mean of all $K_{f,oc}$-values was calculated to be 53.1 mL g^{-1} (mean $K_{f,om}$ of 30.8 mL g^{-1}) with a mean 1/n of 0.916. However, due to the large number of values (n=28), the median sorption value is also considered appropriate for exposure modelling. Since the median $K_{f,oc}$ is with 44.1 mL g^{-1} lower than the arithmetic mean ($K_{f,om}$ of 25.6 mL g^{-1}; median 1/n value =0.990), this represents an even more conservative estimate on the bentazone adsorption behaviour.

Table 7.4/14 Adsorption behaviour of bentazone in different soils

Soil	OC [%]	pH (H_2O) [-]	Soil: Water Ratio [-]	K_f original [mL g^{-1}]	P-value S/W*K_f error	$K_{f,oc}$ original [mL g^{-1}]	Recov. [%]	Uncert. [%]	Correction factor [-]	$K_{f,oc}$corr. [mL g^{-1}]	1/n [-]	$K_{f,oc}$ [§] korr. [mL g^{-1}]	1/n [$] [-]	Ref.
Pfungstadt	0.6	7.8[+]	0.2	0.22	0.04	37.1	98*	2.04	0.53	19.5	0.99	19.5	0.99	Redeker, 1978
LUFA	0.5	7.5[+]	0.2	0.24	0.05	46.5	98*	2.04	0.56	26.2	1.13	26.2	1.13	
Neuhofen	2.7	7.7[+]	0.2	0.35	0.07	13.3	98*	2.04	0.69	9.2	1.03	9.2	1.03	EU review report
Briggs (CA)	1.7	4.9[+]	0.2	3.06	0.61	175.6	100	0	n.r.	175.6	0.70	175.6	0.70	
Monticeller (IL)	1.8	6.0[+]	0.2	0.42	0.08	23.4	100	0	1.00	23.4	0.66	23.4	0.66	Keller, 1986
Greenville (MS)	0.7	7.1[+]	0.2	0.18	0.04	25.2	100	0	1.00	25.2	0.56	25.2	0.56	
Renvill (MI)	2.9	8.2[+]	0.2	0.38	0.08	13.2	100	0	1.00	13.2	0.70	13.2	0.70	EU review report
Pope Farm (NC)	0.6	5.6[+]	0.2	0.45	0.09	77.6	100	0	1.00	77.6	0.70	77.6	0.70	
Mellby (Sweden)	5.9	6.8[+]	0.2	2.90	0.58	49.2	-	-	n.r.	49.2	0.80	49.2	0.80	Bergström et al., 1994 II A 7.4.1/1
Vredepeel (NL)	3.0	5.8[+]	2.0	0.19	0.39	6.4	-	-	n.r.	6.4	0.97	6.4	0.97	Keller, 1995 II A 7.4.1/2
Speyrer Wald	0.7	6.6[+]	1.0	0.02	0.02	3.0	100	0	1.00	3.0	0.85	3.0	0.85	Seher, 1999 II A 7.4.1/3
Borstel	1.2	6.3[+]	1.0	0.07	0.07	5.9	100	0	1.00	5.9	0.98	5.9	0.98	
Dystic Cambisol	3.5	5.3	0.2	1.90	0.38	55.0	99**	1.01	n.r.	55.0	0.99	55.0	0.99	
Stagnic Cambisol	3.4	6.7	0.2	1.40	0.28	41.0	99**	1.01	0.95	39.1	1.00	39.1	1.00	
Calcaric Regosol2	3.3	8.2	0.2	1.20	0.24	36.0	99**	1.01	0.95	34.1	0.99	34.1	0.99	
Fluvic Gleyic Cambisol	2.8	6.2	0.2	1.50	0.30	54.0	99**	1.01	n.r.	54.0	1.00	54.0	1.00	Boivin, 2003
Eutric Cambisol2	2.1	6.2	0.2	1.30	0.26	60.0	99**	1.01	0.95	57.1	1.00	57.1	1.00	Boivin et al. 2005°
Calcaric Regosol	2.1	7.9	0.2	1.20	0.24	63.0	99**	1.01	0.95	59.7	1.00	59.7	1.00	Boivin et al. 2005[b]
Cambic Calcisol	2.1	8.1	0.2	1.30	0.26	55.0	99**	1.01	0.95	52.3	1.00	52.3	1.00	II A 7.4.1/4
Vertic Stagnic Cambisol	2.0	7.0	0.2	1.20	0.24	63.0	99**	1.01	0.95	59.7	1.00	59.7	1.00	II A 7.4.1/5 II A 7.4.1/6
Cambic Stagnic Vertic Calcisol	1.6	8.0	0.2	1.30	0.26	79.0	99**	1.01	0.95	75.2	1.00	75.2	1.00	
Stagnic Luvisol	1.6	5.9	0.2	1.40	0.28	93.0	99**	1.01	0.95	88.7	1.00	88.7	1.00	

Table 7.4/14 Adsorption behaviour of bentazone in different soils

Soil	OC [%]	pH (H_2O) [-]	Soil: Water Ratio [-]	K_f original [mL g^{-1}]	P-value S/W*K_f error	$K_{f,oc}$ original [mL g^{-1}]	Recov. [%]	Uncert. [%]	Correction factor [-]	$K_{f,oc}$corr. [mL g^{-1}]	1/n [-]	$K_{f,oc}$ [§] korr. [mL g^{-1}]	1/n [$] [-]	Ref.
Fluvic Stagnic Cambisol	0.9	5.8	0.2	1.40	0.28	137.0	99**	1.01	0.95	130.7	1.00	130.7	1.00	
Eutric Cambisol	0.8	6.4	0.2	1.20	0.24	158.0	99**	1.01	0.95	149.8	0.99	149.8	0.99	
Fluvic Cambisol	1.1	5.5	0.2	1.50	0.30	144.0	99**	1.01	n.r.	144.0	0.99	144.0	0.99	
Ultuna-0-20cm CT	2.0	5.3	0.8	0.48	0.40	24.3	99**	1.01	n.r.	24.3	0.85	25.0	0.80	Larsbo et al., 2009 II A 7.4.1/8
Ultuna 0-5 cm RD	2.6	5.6	0.8	0.57	0.47	22.1	99**	1.01	n.r.	22.1	0.78			
Ultuna 10-20 cm RD	1.9	6.7	0.8	0.56	0.46	28.7	99**	1.01	n.r.	28.7	0.77			
Säby -0-20cm CT	2.8	5.6	0.8	0.37	0.31	13.5	99**	1.01	n.r.	13.5	0.85	13.2	0.86	
Säby 0-5 cm RD	3.0	5.7	0.8	0.40	0.33	13.0	99**	1.01	n.r.	13.0	0.86			
Säby 10-20 cm RD	2.5	5.7	0.8	0.34	0.28	13.8	99**	1.01	0.95	13.2	0.86			
Lönnstorp -0-20cm CT	1.8	6.7	0.8	0.32	0.27	17.9	99**	1.01	0.95	17.1	1.11	15.1	0.98	
Lönnstorp 0-5 cm RD	2.1	6.2	0.8	0.44	0.37	20.6	99**	1.01	n.r.	20.6	0.94			
Lönnstorp 10-20 cm RD	1.8	6.4	0.8	0.15	0.12	8.3	99**	1.01	0.91	7.5	0.88			
Mean												53.1	0.916	
Median	**Modelling endpoints**											**44.1**	**0.99**	

[+] pH-values are given as pH($CaCl_2$)-values. Transfer to pH(H_2O)-values is done via recommendation of FOCUS 2009: pH(H_2O) = pH($CaCl_2$) * 0.953+0.85
* Recovery was not given in report. Since the study was carried out with radiolabelled material, a recovery of 98% was assumed which represents a worst-case in comparison to the other studies
** Recovery was not explicitly given as percentage in the report/publication. However, it was mentioned that losses did not occur and adsorption to the vial walls and that degradation was tested and found to be not relevant. A value of 99% was chosen to account for remaining uncertainty.
[§] $K_{f,oc}$ and 1/n values of the same soils averaged

Dependency of bentazone sorption from soil properties such as pH-value or OC-content was tested for the parameter selection for modelling and is shown in Section M-III 9.6.1. For this purpose the Kendall test (Kendall's tau coefficient), which is a non-parametric hypothesis test, was used as implemented in the tool Input_Decision 3.1. Statistical significance at 0.05 level was found between the K_f-value and the OC-content whereas no significant correlation was found to the pH-value. Therefore, it is seen justified to use the median $K_{f,oc}$-value as modelling endpoint for exposure assessment.

7.4.2 Adsorption & desorption of rel. metabolites, degr. & react. products

Report:	II A 7.4.2/1 Tornisielo A., Vasques A.C. 2011(b) Adsorption behaviour of BH 351-N-Me (metabolite of BAS 351 H, Bentazone) on different European soils BASF DocID 2011/1021360
Guidelines:	INMETRO NIT DICLA- 035 Rev. 01 (July 2009); OECD 106 (2000); SANCO/825/00 rev. 7 (17 March 2004)
Testing Laboratory and dates:	BASF SA; Guaratingueta; Brazil 12-Mar-2010 - 09-Oct-2010
GLP:	Yes (laboratory certified by Instituto Nacional de Metrologia, Normalizacao e Qualidade Industrial - INMETRO, Rio de Janeiro, Brazil)

Executive Summary

In laboratory experiments the adsorption behaviour of the bentazone metabolite N-methyl-bentazone (Reg. No. 79520) was investigated on three European soils. The three soils covered a range of pH (in $CaCl_2$) from 5.2 to 7.5, a range of organic carbon content from 0.52 % to 1.15 % and two different DIN textural classes: loamy sand and sand.

For the determination of the adsorption isotherm, five different concentrations (nominal 5.0, 2.5, 1.0, 0.5 and 0.05 µg/mL) of the test item in 0.01 M $CaCl_2$ solutions were used. The ratio of soil to test solution was 1/2, and the measurements were performed at the adsorption equilibrium time of 24 hours for all three soils.

The following adsorption parameters were measured for the test item N-methyl-bentazone in each soil: the Freundlich adsorption coefficient $K_{f,}$ the Freundlich exponent 1/n, and the corresponding $K_{f,oc}$ values. The values obtained are summarised in Table 7.4/15.

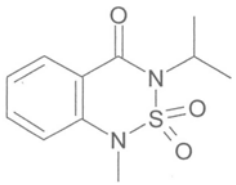
Table 7.4/15 Results from the adsorption experiments with N-methyl-bentazone

Soil	Soil Type (DIN)	Org. C [%]	pH ($CaCl_2$)	K_f [mL/g]	1/n [-]	$K_{f,oc}$ [mL/g]
LUFA 5M	Loamy sand	1.15	7.5	2.354	0.88	204.7
LUFA 2.3	Loamy Sand	1.09	6.9	2.791	0.80	256.1
LUFA 2.1	Sand	0.52	5.2	1.620	0.92	311.6

I. MATERIAL AND METHODS

1. Test Material

Structural formula



Reg. No.	79520
Internal Metabolite Code	BH 351-N-Me
Batch No.	L75-7
Chemical name	3-isopropyl-1-methyl-2,1,3-benzothiadiazin-4(3H)-one 2,2- dioxide
Molecular formula	$C_{11}H_{14}N_2O_3S$
Molecular Weight	254.3 g/mol
Chemical Purity	99.6 %
Expiration Date	September 01, 2014

2. Soils

The study was conducted with three different soils originating from Germany. The physico-chemical characterisation of the soils is provided in Table 7.4/16.

Table 7.4/16 Characterisation of soils used to investigate the adsorption behaviour of N-methyl-bentazone

Soil designation Origin	LUFA 5M	LUFA 2.3	LUFA 2.1
Textural class (German scheme, DIN 4220)	Loamy sand	Loamy sand	Sand
Soil texture [%] (German scheme) % Sand % Silt % Clay	 52.1 34.7 13.2	 54.8 34.0 11.2	 88.2 8.9 2.9
Textural class (USDA)	Sandy loam	Sandy loam	Sand
Soil texture [%] (USDA) % Sand % Silt % Clay	 56.0 30.7 13.2	 56.8 32.0 11.2	 89.1 8.0 2.9
Organic carbon [%]	1.15	1.09	0.52
CEC [$cmol^+/kg$]	13.1	10.4	2.0
pH (water)	8.4	7.9	6.3
pH ($CaCl_2$)	7.5	6.9	5.2

The soils used were sieved to a particle size <2 mm and air-dried at ambient temperature. The actual water content of the soils was determined using a halogen dryer (HB43 Halogen from Mettler Toledo). The residual water content for the adsorption test was between 0.31 % and 0.94 %, which was taken into account for all calculations.

2. Study Design

Experimental conditions

Tests were carried out in duplicate at 20.0 ± 2 °C, in the dark.

A preliminary experiment was run with two soils to find the optimal soil/solution ratio for the adsorption/desorption tests. LUFA 5M and LUFA 2.1 soils were selected to cover the whole pH ($CaCl_2$) range from 5.2 to 7.5 of the present soils. Three different soil/solution ratios were tested (1/1, 1/2 and 1/5), using a $CaCl_2$ solution with a nominal application rate of 5.0 mg/L N-methyl-bentazone. Therefore, samples with 5 g of soil and 5 mL, 10 mL or 25 mL of solution were shaken in teflon centrifugation tubes for 24 h, followed by determination of concentrations in the aqueous phase.

To determine the incubation time needed to reach equilibrium conditions, duplicate samples of each of the three soils were prepared, adding an application solution with a nominal concentration of 5 mg/L to the soils. The experiments were run with a soil/solution ratio of 1/2. In teflon tubes with a capacity of 30 mL, 5 g of soil and 10 mL solution were shaken on a mechanical shaker for 4, 8, 16, 24, 32 and 48 hours at 250 rpm in a temperature controlled dark room at 20 ± 2 °C. The soil/solution suspension was then centrifuged at 5000 rpm for 10 min and the supernatant was removed for analysis. Supernatant aliquots were diluted 1:500 with methanol/water (1/1, v/v). Control runs of all soils were carried out to check for matrix interferences using the same procedure as described above, with untreated soils and $CaCl_2$ solution in centrifugation tubes. The extracts from these runs were also used for the fortification experiments.

Adsorption to the container material was investigated by filling one glass and one teflon tube with 10 mL of a 0.05 µg N-methyl-bentazone /mL solution and analysis of the solution by LC-MS/MS after shaking for 24 h.

The stability of N-methyl-bentazone in solution was investigated by determining the mass balances for all soils and for a 1.0 µg/mL standard solution, after agitation of the samples for 24 hours in glass tubes.

Fortification experiments in solution were performed both on $CaCl_2$ supernatant from control samples and on control soils. Aliquots of 0.95 mL of $CaCl_2$ supernatant were fortified with 0.05 mL of solutions at concentrations of 0.100 µg/mL and 100 µg/mL, resulting in concentrations of 0.005 µg/ml and 5.0 µg/mL, respectively.
In addition, untreated soil samples were fortified with N-methyl-bentazone. Control soil samples of 5 g were fortified with 0.05 mL of solutions in the concentrations of 1.0 µg/mL and 1000 µg/mL, resulting in concentrations of 0.01 mg/kg and 18.0 mg/kg. The soil samples were then extracted and centrifuged the same way as the samples of the adsorption experiments. All fortification samples were determined in triplicate by HPLC-MS/MS.

For the Freundlich adsorption isotherm determinations, solutions of the test item in 0.01 M $CaCl_2$ were prepared with nominal concentrations of 5.0, 2.5, 1.0, 0.5 and 0.05 µg/mL. A soil/solution ratio of 1/2 and an equilibrium time of 24 h were found to be appropriate. Therefore, 5 g samples of the test soil were shaken for 24 hours with 10 mL of the test solution, in glass tubes. Centrifugation was at 3000 rpm for 10 min and the supernatant was collected for analysis, taking into account the weight of the soil and the remaining solution.

The remaining soil in the tubes was extracted with 50 mL of methanol/water 1/1 (v/v) solution, by shaking for 60 minutes at 250 rpm followed by centrifugation for 10 minutes at 3000 rpm. The supernatants were then filtered with 0.2 µm teflon filters.
Analysis of initial solutions, supernatants and soil extracts, diluted to appropriate extent with methanol/water (1/1, v/v) was performed by LC-MS/MS.

Desorption experiments were not conducted since the soil was extracted after the adsorption phase with organic solvent.

Description of analytical procedures

The method of analysis was validated for the aqueous phases after adsorption, using control samples of all three soils.

Soil and 0.01 M $CaCl_2$ in the ratio 1/1 were weighed in centrifuge tubes, shaken for 24 hours and then centrifuged. After centrifugation, the supernatant was used for fortification experiments at the LOQ (0.005 µg/mL) and at the highest nominal test concentration (5 µg/mL), including one unfortified control sample per soil.

The limit of quantitation (LOQ) for the $CaCl_2$ supernatant was 0.005 µg/ml, corresponding to 10 % of the lowest nominal test concentration.

The LOQ for the soil extracts was 0.01 mg/kg dry soil, using a previously validated method (method L0136/01).

II. RESULTS AND DISCUSSION

1. Preliminary tests

The soil/solution ratio of 1/2 resulted in a mean adsorption value of 55.8 % for LUFA 5M soil and 46.0 % for LUFA 2.1 soil. At the ratio 1/1, the mean adsorption was above 60.0 % for both soils and, at 1/5, under 35.0 %. Therefore a soil / solution ratio of 1/2 was considered to be appropriate for the isotherm experiments, because it allowed a reasonable amount of test item remaining in both aqueous and extract phases for accurate measurements.

The adsorption equilibrium test revealed that the equilibrium conditions were reached after 24 hours with all three soils. Adsorption values were 60.8 % for the LUFA 5M soil, 62.9 % for the LUFA 2.3 and 50.5 % for the LUFA 2.1 soil after 24 h.

To test the adsorption to the container material, recovery of N-methyl-bentazone was measured from glass and teflon tubes without soil. Mean recovery rates of 99.7 % and 91.3 % for the adsorption test in glass and teflon tubes, respectively, showed that glass was the appropriate material to conduct this study.

During the stability test, the recovery rates for the 1.0 µg/mL solution were under 90 % for some of the replicates, based on the direct method, and under 70 % for all soils based on the indirect method. For these reasons, the direct method was chosen for the conduction of the adsorption isotherms determination.

The recovery rates of the lowest level of sample fortification prepared from control solution ranged from 87 % (LUFA 2.1 soil) to 111 % (LUFA 2.3 soi), both on the stability test. The recovery rates of the highest level ranged from 86 % (LUFA 2.3 soil) on the soil / solution ratio test to 109 % (LUFA 5M soil) on the equilibrium test. These results confirm that the methodology was well suited to determine residues of N-methyl-bentazone in $CaCl_2$ solution in the presence of soil components, which were co-extracted under the applied conditions. The recovery rates of the lowest and highest levels in spiked soils extracts ranged, respectively, from 81 % (LUFA 2.3 soil) to 110 % (LUFA 2.1 soil) and from 89 % (LUFA 2.3 soil) to 108 % (LUFA 5M soil). All the extremes presented were from the stability test. These results confirm that the methodology was well suited to determine residues of N-methyl-bentazone in organic soil extracts.

Method validation: The analytical method for the determination of N-methyl-bentazone in the $CaCl_2$ aqueous phase was developed within this study. To demonstrate the efficiency of the method, aliquots of all three soils were shaken with untreated $CaCl_2$ and the supernatants subsequently spiked with N-methyl-bentazone. At the highest concentration of 5 µg/mL, recoveries between 86 % (LUFA 2.3 soil) and 102 % (LUFA 5M and LUFA 2.1 soils) were obtained for the $CaCl_2$ supernatant. At the concentration of 0.005 µg/mL (LOQ), the recoveries ranged from 91 % to 106 % (both LUFA 5M soil). The average overall recovery (both concentration levels and all soils) was 96 %. Control measurements without test item proved the specificity of the analytical method since no signals originating from matrix constituents were observed.

2. Freundlich adsorptions isotherm determination

The Freundlich adsorption coefficients K_f covered a range from 1.620 (LUFA 2.1 soil) to 2.791 mL/g (LUFA 2.3 soil) for the three soils. The $K_{f,oc}$ values ranged from 204.7 mL/g (LUFA 5M soil) to 311.6 mL/g (LUFA 2.1 soil), with the Freundlich adsorption exponent ranging from 1/n = 0.80 (LUFA 2.3 soil) to 0.92 (LUFA 2.1 soil). The results are summarised in Table 7.4/17.

Table 7.4/17 Results from the adsorption experiments with N-methyl-bentazone

Soil	Soil Type (DIN)	Org. C [%]	pH ($CaCl_2$)	K_f [mL/g]	1/n [-]	$K_{f,oc}$ [mL/g]
LUFA 5M	Loamy Sand	1.15	7.5	2.354	0.88	204.7
LUFA 2.3	Loamy Sand	1.09	6.9	2.791	0.80	256.1
LUFA 2.1	Sand	0.52	5.2	1.620	0.92	311.6

3. Mass balance

A mass balance determination was carried out for all soils and all concentrations from the Freundlich adsorption isotherm determination. For test item N-methyl-bentazone, the values for the recovery ranged from 96.2 % to 106.4 % for LUFA 5M soil, 81.6 % to 105.1 % for LUFA 2.3 soil and 96.5 % to 107.4 % for LUFA 2.1 soil.

III. CONCLUSION

The adsorption behaviour of N-methyl-bentazone was determined on three European soils, using the direct method (determination of compound concentrations in both aqueous and soil phase). The soils covered a range of pH (in $CaCl_2$) from 5.2 to 7.5, a range of organic carbon content from 0.52 % to 1.15 % and two different DIN textural classes: loamy sand and sand. The Freundlich adsorption coefficient K_f covered a range from 1.620 mL/g to 2.791 mL/g for the three soils. The $K_{f,oc}$ values ranged from 204.7 mL/g to 311.6 mL/g.

7.4.3 Column leaching studies with the active substance

Column leaching studies of bentazone were evaluated and peer-reviewed during last Annex I inclusion. No additional studies have been performed. A brief summary of the reviewed data is provided below.

In a column leaching study, the mobility of bentazone was studied in three German standard soils (BBA 2.1, BBA 2.2 and BBA 2.3). In all soils, up to 100% bentazone were retrieved from the percolation water.

In an aged residue column leaching study, the mobility of bentazone was studied. Bentazone was aged for 30 days before transfer of the soil to the columns. 18 – 67 % bentazone were retrieved from the percolation water, together with 7% undefined polar products.

The low adsorption of bentazone on these soils indicated that risk of groundwater contamination could not be excluded.

7.4.4 Column leaching studies rel. metabolites, degr. & and react. products

See chapter 7.4.3.

7.4.5 Aged residue column leaching

See chapter 7.4.3.

7.4.6 Leaching (TLC)

Not performed - not considered necessary.

7.4.7 Lysimeter studies

Lysimeter studies with bentazone were provided during last Annex I inclusion and were already peer-reviewed. No new lysimeter studies were performed.

Overall, four lysimeter studies including in total 7 undisturbed soil cores with duration of 2 to 3 years were conducted in Germany and carried out according to the Germany lysimeter guideline. Radiolabelled bentazone was applied either to winter wheat, spring wheat or peas in different sandy worst-case soils with an application rate of 0.5 to 1.5 kg a.s. ha^{-1} in an application frequency of one to two applications. The maximum concentrations observed in leachate samples during the study period was 0.027 to 0.178 µg active substance/L with average annual concentrations of 0.004 to 0.085 µg active substance/L.

According to these results, bentazone is not expected to exceed the trigger value of 0.1 µg L^{-1} when used according to good agricultural practice.

7.4.8 Field leaching studies

Since several lysimeter studies are available, no field leaching study was performed for bentazone.

7.4.9 Volatility - laboratory study

Based on its physical-chemical properties, bentazone has no potential for volatilisation (4.9×10^{-6} Pa at 20°C). Furthermore, volatilisation from moist soil surfaces is not expected to be an important process for bentazone based on a Henry's law constant of 2.108 10 kPa m³/mol. Calculation of the atmospheric half-life shows that bentazone will be degraded by reaction with hydroxyl radicals with an estimated half-life of 2.1 hours. A long-range transport via air can therefore be excluded. Volatility studies are thus not considered necessary.

7.5 Hydrolysis rate of relevant metabolites at pH values 4, 7 and 9

The hydrolysis of bentazone was evaluated and peer-reviewed already during the last Annex I inclusion procedure. Bentazone proved to be stable at all pHs tested. No new hydrolysis study was performed.

7.6 Direct phototransformation of relevant metabolites in water

Report:	II A 7.6/1 Singh M. 2011(a) Aqueous photolysis of 14C-BAS 351 H BASF DocID 2011/7002318
Guidelines:	EPA 835.2240; EPA 161-2; OECD Guideline for Testing of Chemicals; FAO Revised Guidelines on Environmental Criteria for the Registration of Pesticides Revision 3 (28 August 1993); OECD 316 (Photodegradation in Water)
Testing Laboratory and dates:	BASF Agricultural Research Center; Research Triangle Park NC; United States of America 20-Mar-2009 - 20-Jul-2009
GLP:	Yes (laboratory certified by United States Environmental Protection Agency)

Executive Summary

The aqueous photolysis of ^{14}C-BAS 351 H (^{14}C-bentazone, phenyl label) was investigated in buffer solutions pH 5, 7 and 9 at a temperature of 22 ± 1°C. The concentrations of BAS 351 H in the buffer solutions were 17.8 mg/L (pH 5 and 7) 16.7 mg/L (pH 9). The treated solutions were continuously exposed to artificial sunlight (filtered Xenon lamp) in an Atlas Suntest CPS Plus apparatus for about 15 days. The measured intensity and emitted light spectrum of the xenon lamp were comparable to natural sunlight at 40° N latitude. During irradiation, CO_2-free sterile air was purged over the samples and through the NaOH traps.

Duplicate treated samples, (irradiated and dark control) were analysed concurrently by LSC and HPLC after about 0, 3 (4), 6, 10 (11), 13, and 15 days after treatment (DAT).

For determination of the quantum yield of BAS 351 H, a mixture of p-nitroacetophenone (PNAP) and pyridine was used as chemical actinometer. The vessel with the actinometer solution was irradiated under similar conditions as the bentazone treated test vessels.

The material balance for the irradiated samples ranged from 92.8 - 101% of the total applied radioactivity (TAR). About 10.4 - 11.7% TAR (pH 5), 6 - 6.7% TAR (pH 7) and 2.5 - 4.5% TAR (pH 9) were found as cumulative volatile radioactivity at the end of the photo-period in the volatile traps. All volatile radioactivity was identified as $^{14}CO_2$. The material balance for the dark control samples ranged from 96.8 - 102% TAR.

Bentazone degraded fast at all pH values to about 4.2-14.4% TAR during 15 days irradiation. Fifteen to seventeen metabolites were observed at 15 DAT. Bentazone, Peak B, Peak C (sulfonic acid) and one very polar unknown peak (UNK ~2.5 min) were the major radioactive residues (≥ 10% TAR) at various sampling intervals. The degradation product PeakB reached max. amounts of about 8.5-29.9% TAR. The degradation product PeakC (sulfonic acid) reached about 20.2 – 25.0% at 15 DAT. The peak UNK 2.5/2.6 was found in amounts of 5.7 – 29.5 %TAR at 15 DAT. UNK 2.5/2.6 was very polar and was shown to consist of multiple components. Other degradation products in amounts between 5 and 10% TAR were only found in buffer pH 5 (UNK 7.3 accounting for 5.7% TAR at 15 DAT) or buffer pH 9 (UNK 3.5 and UNK 17.5 accounting for 5.1% and 8.2% at 15 DAT). All other degradation products were minor and did not exceed 4.9% TAR.

In the dark control, the parent bentazone was the only major radioactive residue present at all sampling intervals.

Peak identification of bentazone, "PeakB", "PeakC" and a minor degradation product BH 351-AIPAM was achieved using HPLC co-chromatography with reference compounds and/or mass spectrometry.

The calculated half-lives of bentazone under photolytical conditions were 3.3 days at pH 5, 5.4 days at pH 7 and 3.9 days at pH 9.

Furthermore environmental photolytic half-lives were calculated considering the quantum yields of BAS 351 H determined in this study.

I. MATERIAL AND METHODS

A. MATERIALS

1. Test Material	[U-^{14}C-phenyl]-BAS 351 H
Specific radioactivity	5.23 MBq/mg (317400 dpm/µg)
Lot/Batch #:	210-2201,
radiochemical purity	97.3%
CAS #:	25057-89-0
Molecular weight:	240.28 g/mol (unlabelled)
Solubility in water:	570 mg/L at 20°C

2. Buffer solutions:
The test systems for this study were aqueous buffer solutions of pH 5, 7 and 9. Sodium acetate was used for the preparation of pH 5 buffer solution. TRIS (hydrochloride) and TRIS (base) were used for the preparation of pH 7 and pH 9 test buffer solutions (0.01 M), respectively. The buffer solutions were autoclaved at 120°C before use.

B. STUDY DESIGN

1. Experimental conditions

The photolysis test was performed at concentrations of 17.8 mg/L ^{14}C-BAS 351 H for pH 5 and pH 7 buffer, and 16.7 mg/L ^{14}C-BAS 351 H for the pH 9 buffer.

Photolysis was carried out in an Atlas Suntest CPS Plus unit with a xenon lamp emitting sunlight similar spectrum equipped with filters to cut-off wavelengths <290 nm. Continuous irradiation was employed throughout the study. The light intensity in the wavelength range between 300 - 800 nm was measured using a LI-COR model LI-1800 Spectroradiometer at beginning and end of the study. The average light intensity (pre- and post- study) was 618 W/m^2, comparable to natural sunlight at 40° N latitude.

The photolysis setup consisted of a rectangular metallic hollow block equipped with a coolant inlet and outlet. The thermostated block was provided with six wells to house six photolysis glass vessels. Each glass vessel had an air inlet and outlet and could hold ~275 mL solution. Each photolysis vessel was provided with a quartz glass disc at the top.

One vessel was filled with ethylene glycol/water mixture (1:1) and was placed into one of the wells. A thermocouple probe was inserted into this mixture to measure the temperature during the photo-period. One vessel was filled with actinometer solution and was placed into one of the wells. Two vessels were filled with 200 mL ^{14}C-BAS 351 H treated pH buffer solution and were placed in wells on the block. The two vessels containing the test substance served as separate replicates. Each vessel was connected independently to trapping solutions for the collection of volatile radioactivity.

During the actual irradiation period, a stream of sterile, moistened and CO_2-free air was purged through the vessels under negative pressure. Air exiting the reaction vessels was successively passed through two 1 N NaOH traps (~20 mL) for collection of $^{14}CO_2$. The coolant was circulated through the metallic block to maintain the test solutions at 22 ± 1 °C. At each sampling interval an aliquot (~1 mL) was removed from each vessel for LSC and HPLC analysis. Volatile trapping solutions were removed at every sampling interval and were analysed by LSC to estimate the amount of volatile radioactivity.

Dark control samples in HPLC vials consisting of 1 mL buffer aliquots (pH 5, 7 and 9) treated with ^{14}C-phenyl-labeled BAS 351 H were stored in a dark incubator maintained at 22 ± 1°C.

For determination of the quantum yield of BAS 351 H, a mixture of p-nitroacetophenone (PNAP, 2.6 x 10^{-5} M) and pyridine (2 x 10^{-2} M) was used as chemical actinometer. The vessel with the actinometer solution was irradiated under similar conditions as the other test vessels.

Sterility checks were performed for the experimental and actinometer solutions at starting and end points of incubation. The test systems were under sterile conditions for the entire experimental period.

2. Sampling

Irradiated and dark control samples were removed at the same time for LSC and HPLC analyses. The sampling intervals for the pH 5 and 7 test systems were 0, 3, 6, 10, 13 and 15 days after treatment. The sampling intervals for the pH 9 test system were 0, 4, 6, 11, 13 and 15 days after treatment.

3. Description of analytical procedures

The irradiated samples were analysed concurrently with the dark control samples. Aliquots (3 x 10 µL) of the ^{14}C-BAS 351 H treated samples (irradiated samples and dark controls) were assayed by LSC at every sampling interval to determine the material balance.
A 40 µL aliquot of each sample was analysed by HPLC to obtain the quantitative residue distribution profile for every sampling interval.

The radioactivity in the NaOH trapping solutions was determined by LSC (3 x 0.5 or 1 mL). The radioactive volatiles in the NaOH solution were confirmed to be CO_2 by adding sulfuric acid to an aliquot of the trapping solution, trapping the evolving $^{14}CO_2$ in a Harvey scintillation cocktail, and finally counting the sample by LSC.

The irradiated actinometer sample was analysed for PNAP at each sampling interval by HPLC-UV (270 nm). The HPLC-UV peak area of PNAP at a given time point was compared to that at time zero. The relative peak area was expressed as a percentage and was used for the half-life calculation of the actinometer.

Isolation and identification of the ^{14}C degradation products

pH 5 test system
Aliquots of 6 DAT photolysis samples (1.2 mL, 1:1 mixture of Rep 1 and Rep 2) were analysed by LC/MS/MS before and after concentration. For concentration, the sample was evaporated to dryness under a stream of N_2. The residual material was dissolved in a mixture of water/methanol (2:1, 60 µL) by sonication.

40 µL aliquots of the 6 DAT photolysis sample were injected separately with reference standards of BAS 351 H (parent), ^{14}C-PeakB and ^{14}C-PeakC (sulfonic acid).

pH 9 test system
The photolysis samples remaining at the end of the study (Rep 1 and Rep 2) were mixed and lyophilized. The residual solid was dissolved in a mixture of methanol/water (13 mL, 40:60) by sonication. An aliquot of the sample was analysed by LC/MS/MS. The lyophilized sample prepared above was used for HPLC fraction collection for the isolation of the polar peak (~2.5 min) and the 17.5 min peak.

Half-life calculations

Estimation of the half-life of bentazone based on the %TAR values for BAS 351 H at various sampling intervals was done only for the irradiated samples, since it proved to be stable under dark conditions. Half-life calculations were based on the assumption that photolysis degradation followed first order kinetics.

The quantum yield of BAS 351 H was calculated according to the method of Dulin and Mill using the half-life of the actinometer, the DT_{50} of the parent compound in the irradiated system, the UV-absorbance profiles of the actinometer and the experimental solutions, and the irradiation energy data for the Atlas Suntest provided by the manufacturer.

II. RESULTS AND DISCUSSION

A. MATERIAL BALANCE

The recovery of radioactivity in the irradiated and dark control samples as well as the amount of radioactivity in the volatile traps is shown in Table 7.6/1. The material balance for the irradiated test systems ranged from 92.8 – 101 %TAR, 93.8 - 100 %TAR and 94.4 - 100 %TAR for pH 5, pH 7 and pH 9 buffer solutions, respectively. The material balance for the dark controls ranged from 97.9 – 101 %TAR, 96.8 – 100 %TAR and 99.8 - 102 %TAR for pH 5, pH 7 and pH 9 buffer solutions, respectively. The volatile radioactivity increased with time for all the test systems.

Photolysis in pH 5 produced the largest amount of volatile radioactivity and was approximately 11.1% TAR at 15 DAT (average of two replicates). Photolysis in pH 9 produced the lowest amount of volatile radioactive residues and was approximately 3.5% TAR at 15 DAT (average of two replicates). All volatile radioactivity was identified as $^{14}CO_2$. The radioactivity remaining in the NaOH trapping solution after acidification was below the limit of quantitation.

BAS 351 H was stable in test buffer solutions under dark conditions. No attempt was made to estimate volatile radioactivity in the dark control samples.

Table 7.6/1 Recovery of radioactivity during aqueous photolysis of [U-^{14}C-phenyl]-BAS 351 H; mean of two replicates [% TAR]

days after treatment	pH 5 Cumulative Volatile	pH 5 Material balance	pH 7 Cumulative Volatile	pH 7 Material balance	pH 9 Cumulative Volatile	pH 9 Material balance
irradiated						
0		100		99.5		99.9
3/4	1.0	97.9	0.65	96.4	0.21	99.3
6	2.7	97.3	1.9	96.9	0.54	98.9
10/11	6.2	94.4	3.7	95.0	1.9	97.9
13	9.0	93.6	5.2	94.2	2.7	95.6
15	11.1	93.6	6.3	94.3	3.5	97.2
dark control						
0	-	100	-	99.5	-	99.9
3/4	-	98.3	-	97.2	-	101
6	-	99.3	-	97.7	-	101
10/11	-	98.6	-	98.1	-	101
13	-	99.6	-	98.3	-	101
15	-	99.2	-	97.8	-	100

B. FINDINGS

The distribution of radioactivity in the water phases is summarized in Table 7.6/2 to Table 7.6/5.

In all irradiated test sytems, BAS 351 H degraded to a number of products. For all pH values tested, the number of the degradation products increased with time.

The HPLC analysis of the dark control revealed only one major radioactive residue at every sampling interval (BAS 351 H: t_R = ~18.3). The amount of BAS 351 H ranged from 95.2 to 97.9%, 94.5 to 96.0% and 97.7 to 99.5% TAR for pH 5, pH 7 and pH 9 test systems, respectively, during the course of the study (average of two replicates given).

pH 5
After 15 days continuous irradiation, a total of 15 radioactive residues could be detected by HPLC in the sample of the pH 5 buffer solution. The majority of these radioactive residues were formed after the 6 DAT sampling interval. BAS 351 H, PeakB, and PeakC (sulfonic acid) were found to be the major radioactive residues. The amount of BAS 351 H decreased with time from 97.9% TAR at the beginning to 4.2 %TAR at the end of the study (averages of two values). The degradation product PeakB increased with time and accumulated to 26.3% TAR (average of two replicates) by the final sampling interval. The degradation product PeakC (sulfonic acid) also increased over time and accounted for 20.2% TAR at 15 DAT (average of two replicates). The degradation product UNK 2.6 reached a maximum amount of 5.7%TAR. This peak was isolated and further characterized during the pH 9 photolysis experiment and could be shown to be a mixture of multiple components. The degradation product listed as “Others” was a mixture of multiple components and none of the individual components exceeded 4.8%TAR. One of these components was identified as BH 351 AIPAM.

pH 7
The HPLC analysis results of 15 DAT sample of the pH 7 buffer revealed a total of 17 radioactive residues. The majority of these radioactive residues were formed after the 6 DAT sampling interval. BAS 351 H, PeakB, PeakC (sulfonic acid) and UNK 2.5 were found to be the major radioactive residues during the study duration. The amount of BAS 351 H decreased with time from 96.0 %TAR at the beginning to 14.4 %TAR at the end of study (average of two replicates). The degradation product PeakB increased with time and accumulated to a maximum of 16.8% TAR (average of two replicates) by the final sampling interval. The degradation product PeakC (sulfonic acid) also increased over time and accounted for a maximum of 25.2% TAR at 13 DAT. The very polar degradation product UNK 2.5, increased to 10.0% TAR at 15 DAT. The degradation product listed as “Others” was a mixture of multiple components and none of the individual components exceeded 4.9%TAR. One of these components was identified as BH 351 AIPAM.

pH 9

The HPLC analysis results of 15 DAT sample of the pH 9 buffer system revealed a total of 17 radioactive residues. BAS 351 H, PeakB, PeakC (sulfonic acid), UNK 17.5 and UNK 2.5 min were found to be the major radioactive residues during the study duration. The amount of BAS 351 H decreased with time from 97.7 %TAR at the beginning to 8.8 %TAR at the end of the study. The degradation product PeakB slowly increased over time and accumulated to a maximum of 8.5% TAR (average of two replicates) by the final sampling interval. The degradation product PeakC (sulfonic acid) also increased to a maximum of 23.7% TAR at 10 DAT and then began to decline slowly (15 DAT: 22.8 % TAR). The degradation product UNK 17.5 accumulated to a maximum of 9.9% TAR (10 DAT) and then began to decline. This degradation product was found to be a mixture of several components. The very polar degradation product UNK 2.5 increased to about 29.5% TAR at 15 DAT. This degradation product was found to be a mixture of several components. The degradation product listed as "Others" was a mixture of multiple components and none of the individual components exceeded 4.8%TAR.

Table 7.6/2 HPLC quantitation of ^{14}C-residues in the dark controls treated with [U-^{14}C-phenyl]-BAS 351 H; mean of two replicates [% TAR]

	pH 5		pH 7		pH 9	
	BAS 351 H t_R ~18.3 min	Others[1]	BAS 351 H t_R ~18.3 min	Others[1]	BAS 351 H t_R ~18.3 min	Others[1]
0	97.9	2.5	96	3.5	97.7	2.2
3	95.3	3.1	94.6	2.6	99.3	1.6
6	95.8	3.6	94.6	3.2	98.8	2.0
10	95.2	3.4	95	3.2	99.2	1.9
13	96.1	3.6	95.3	3.1	99.3	2.0
15	96.4	3.0	94.5	3.4	99.5	1.0

[1] Sum of multiple components, none totalling more than 1.3% TAR
t_R = retention time

Table 7.6/3 HPLC quantitation of ^{14}C-residues in the irradiated test system pH 5 treated with [U-14C-phenyl]-BAS 351 H; mean of two replicates [% TAR]

DAT	UNK 2.6[1] (t_R ~ 2.6 min)	UNK 7.3 (t_R ~ 7.3 min)	Peak B (t_R ~ 8.2 min)	Peak C (t_R ~ 15.8 min)	BAS 351 H t_R ~18.3 min	Others[2]
0	-	-	-	-	97.9	2.4
3	-	1.3	13.7	8.7	67.9	5.3
6	-	3.2	24.4	14.7	41.4	10.9
10	3.0	4.4	29.9	19.7	17.0	14.3
13	4.9	5.6	29.7	21.1	7.5	16.0
15	5.7	5.7	26.3	20.2	4.2	20.5

- not detectable
[1] 2.6 minute peak was very polar and a mixture of several components
[2] Sum of multiple components, no single peak exceeds 4.8% TAR
t_R = retention time

Table 7.6/4 HPLC quantitation of ^{14}C-residues in the irradiated test system pH 7 treated with [U-^{14}C-phenyl]-BAS 351 H; mean of two replicates [% TAR]

DAT	UNK 2.5[1] (t_R ~ 2.5 min)	Peak B (t_R ~ 8.2 min)	Peak C (t_R ~ 15.8 min)	BAS 351 H t_R ~18.3 min	Others[2]
0	-	-	-	96	3.5
3	1.4	4.3	8.2	77.7	4.2
6	3.0	9.8	15.3	57.6	8.9
10	6.6	14.3	23.0	32.1	15.5
13	7.9	16.7	25.2	20.6	18.8
15	10.0	16.8	25.0	14.4	21.8

- not detectable
[1] 2.5 minute peak is very polar and a mixture of several components
[2] Sum of multiple components, no single peak exceeds 4.8% TAR
t_R = retention time

Table 7.6/5 HPLC quantitation of ^{14}C-residues in the irradiated test system pH 9 treated with [U-^{14}C-phenyl]-BAS 351 H; mean of two replicates [% TAR]

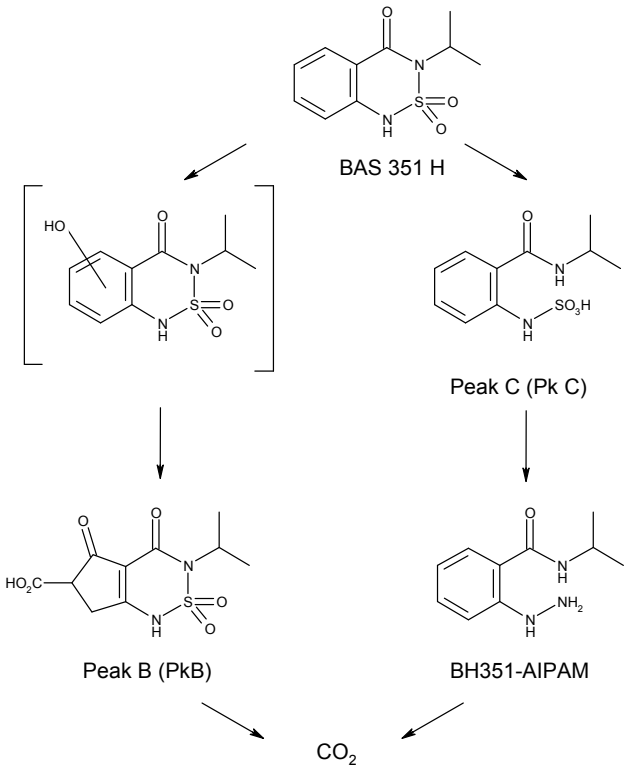
DAT	UNK 2.5[1] (t_R ~ 2.5 min)	UNK 3.5 (t_R ~ 3.5 min)	Peak B (t_R ~ 8.2 min)	Peak C (t_R ~ 15.8 min)	UNK 17.5[1] (t_R ~ 17.5 min)	BAS 351 H t_R ~18.3 min	Others[2]
0	-	-	-	-	-	97.7	2.2
3	8.8	0.7	3.6	12.7	4.9	65.9	2.4
6	14.0	1.6	5.3	19.2	8.2	44.7	5.7
10	24.7	6.1	7.6	23.7	9.9	14.8	9.5
13	27.5	5.2	7.5	21.7	8.5	10.3	12.5
15	29.5	5.1	8.5	22.8	8.2	8.8	10.9

- not detectable
[1] Peaks at ~2.5 minutes and ~17.5 minutes were a sum of multiple components
[2] Sum of multiple components, no single peak exceeds 4.8% TAR

Proposed Photolytic Degradation Pathway

The photolytic degradation pathway for BAS 351 H is shown in the Figure 7.6/1. BAS 351 H degraded quickly in buffer solutions of pH 5, 7 and 9 under photolytic conditions. The major pathways of degradation are: 1) opening of the thiadiazine ring leading to the degradation products PeakC (sulfonic acid) and AIPAM, and 2) hydroxylation of the benzene ring followed by opening of the hydroxylated benzene ring leading to degradation product PeakB. Ultimately, BAS 351 H mineralizes to $^{14}CO_2$.

Figure 7.6/1 Proposed route of degradation of bentazone during aqueous photolysis



Photolytic half-lives

Estimated half-lives and quantum yields are given in Table 7.6/6.

Table 7.6/6 Photolytic half-lives and quantum yield of BAS 351 H in sterile buffer

pH of buffer	DT_{50} [d]	Data Correlation coefficient	Quantum Yield (mol Einstein^{-1})
pH 5	3.3	0.977	7.7×10^{-3}
pH 7	5.4	0.965	4.7×10^{-3}
pH 9	3.9	0.968	6.0×10^{-3}

Figure 7.6/2 Fitted curve of BAS 351 H photolysis in buffer at pH 5

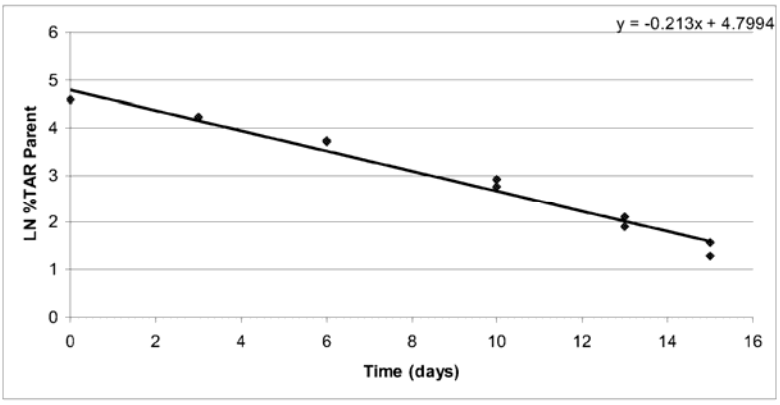


Figure 7.6/3 Fitted curve BAS 351 H photolysis in buffer at pH 7

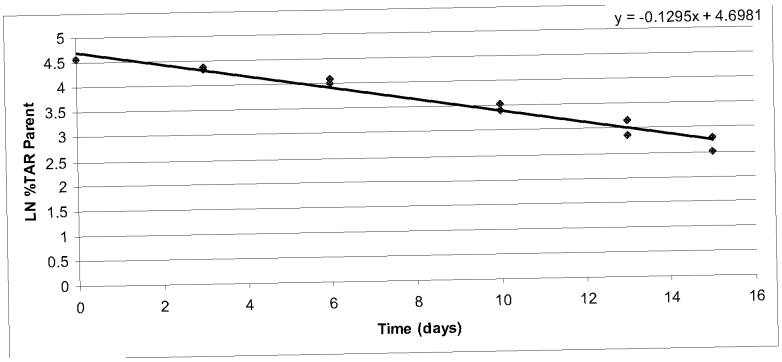
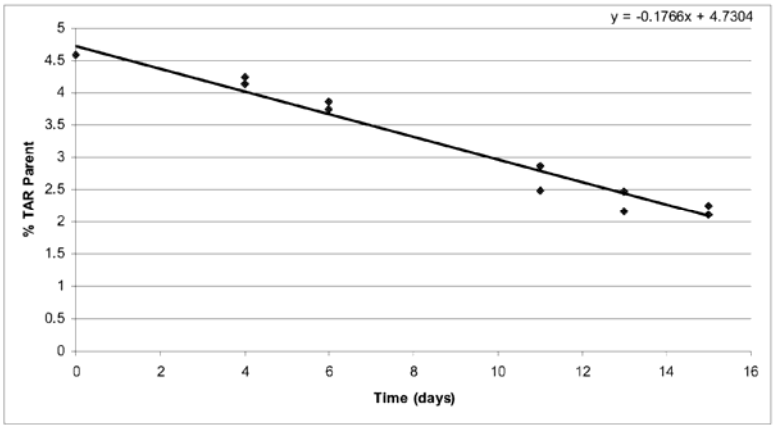


Figure 7.6/4 Fitted curve BAS 351 H photolysis in buffer at pH 9



Calculation of direct phototransformation in water

The theoretical half-lives of BAS 351 H was determined for aqueous solutions at pH 5, 7 and 9.

The theoretical half-lives of BAS 351 H in the top layer of natural aqueous systems were calculated with a program which uses the algorithms developed by Frank and Klöpffer (*Frank, R, and Klöpffer, W., Ermittlung von Strahlundsdaten und Entwicklung eines Programms zur Abschätzung der abiotischen Transformation von Chemikalien in natürlichen Gewässern, Forschungsbericht Nr. 106 020 46, 1985.*) for the direct phototransformation of chemicals in water. This model uses data specific to the test substance, including the quantum yield (Φ_{ts}), and the absorption coefficient ($\varepsilon_{(\lambda)ts}$). The model also uses natural light intensities measured for each month in central Europe. Other inputs to the model included specifying a 1 cm thickness of the upper layer of water, a 10 mg/L (or 10 ppm) water concentration, and 10% losses by reflection. The DT_{50} calculation was made for each month from January to December.

Table 7.6/7 Theoretical photolytical half-lives of BAS 351 H in the top layer of aqueous systems

Month of application	Day length [h]	Theoretical environmental half-life					
		pH 5		pH 7		pH 9	
		DT_{50} [h]	DT_{50} [d]	DT_{50} [h]	DT_{50} [d]	DT_{50} [h]	DT_{50} [d]
April	13.67	1.46	0.11	2.41	0.18	1.76	0.13
May	15.44	1.27	0.08	2.08	0.13	1.53	0.10
June	16.47	1.20	0.07	1.97	0.12	1.44	0.09
July	16.07	1.32	0.08	2.18	0.14	1.59	0.10
August	14.53	1.25	0.09	2.06	0.14	1.51	0.10
September	12.56	1.91	0.15	3.14	0.25	2.30	0.18
October	10.57	3.14	0.30	5.16	0.49	3.77	0.36
November	8.65	6.32	0.73	10.39	1.20	7.60	0.88
December	7.55	11.05	1.46	18.16	2.41	13.28	1.76
January	8.02	7.14	0.89	11.73	1.46	8.58	1.07
February	9.67	3.85	0.40	6.33	0.65	4.63	0.48
March	11.60	2.18	0.19	3.59	0.31	2.63	0.23

III. CONCLUSION

The results showed that bentazone was rapidly degraded in sterile water under photolytic conditions. The degradation half-lives were about 3.3 days, 5.4 days and 3.9 days in pH 5, 7 and 9 buffer solutions, respectively. BAS 351 H was stable in test buffers (pH 5, 7 and 9) under dark conditions.

A rather large number of photo-products was formed (≥ 15), two of them exceeding 10% of the total applied radioactivity. These two peaks were designated as PeakB (max. 30% TAR at pH 5), and PeakC (max. 25% TAR at pH 7). The rest of the degradation products were minor and did not exceeded 5% TAR.

Report:	II A 7.6/2 Housari F.A. et al. 2010(a) Factors responsible for rapid dissipation of acidic herbicides in the coastal lagoons of the Camargue (Rhone River Delta, France) BASF DocID 2011/1276854
Guidelines:	None
Testing Laboratory and dates:	19-Oct-2010
GLP:	No, not subject to GLP regulations (scientific publication)

Executive Summary

The dissipation of three acidic herbicides, among them bentazone, was investigated in water from the Vaccarès lagoon (brackish water) and the Canal de Fumemorte (fresh water) in the Camargue region. For this summary, only the photo-degradation and hydrolysis experiments for bentazone are described.

Biodegradation, direct and indirect photochemical degradation as well as hydrolysis were investigated in both types of water samples by an experimental approach. Elimination by sedimentation, volatilization and flushing was assessed by a reactive transfer model (MASAS - Modelling of Anthropogenic Substances in Aquatic Systems), using meteorological and hydrological data from the studied area.

For photo-degradation the samples were filtrated through 0.45 µm membranes. Experiments were conducted in sunlight under summer conditions in Marseille, France at a bentazone concentration of 50 µM and over study duration of eight days.

It was found that direct (81%) and indirect photolysis (17%) are the main processes of dissipation of bentazone in canal and lagoon water. Half-life times of 2.2 d in canal water and 4.1 d in lagoon water were found for photo-degradation of the substance.

I. MATERIAL AND METHODS

1. Test Material

Bentazone (99.7% purity), was obtained from Riedel-de-Haën. NaN_3 (99%) and acetonitrile (ACN) were from Sigma-Aldrich, 30% w/w hydrogen peroxide solution was from Fischer. The ultra purity water (UHQ) for all experiments and all analyses by HPLC was obtained by a Milli-Q water system.

2. Test Systems

The area from which the two different waters were sampled for this study is the Île de Camargue, the central part of the Rhone delta in the south of France. The Camargue regional nature park lies in the center of the Île de Camargue and covers 85,000 ha of lagoons, marshes and other lands separated from the Mediterranean Sea by a dike. Agricultural land borders the park to the North and South-East, and most of it is devoted to intensive flooded rice cultivation. Runoff from the rice parcels is collected from mid-April to September in canals. One of these canals, the Canal de Fumemorte, discharges into the Vaccarès lagoon which lies in the protected area. Both the water of Fumemorte canal and Vaccarès lagoon were studied here. The water samples represented fresh water (Canal) and brackish water (Vaccarès lagoon). They differ mainly in the type and quantity of organic matter, and in the salinity, which is below 1 g/L in the canal water and about 25 g/L in the Vaccarès lagoon. Two different types of water samples were selected to elucidate if there is any or possible impact of water type on photo-degradation.

3. Study Design

Sampling and sample preparation
Water samples were taken from about 15 cm below the surface (lagoon and canal), filtered through 0.45 µm pore-size membranes, and stored at 4 °C in the dark till analysis. Storage and all measurements were performed at the pH of the natural water. The chloride ion content was $1.18 \pm 0.03 \times 10^{-3}$ M and $1.90 \pm 0.05 \times 10^{-1}$ M in canal water and lagoon water samples, respectively. Non Purgeable Organic Carbon (NPOC) values were 19.8 ± 0.3 and 32.1 ± 0.6 mg C L^{-1} in canal water and lagoon water samples, respectively.

Analytical procedures
The quantification of bentazone was done directly by HPLC chromatograph equipped with a UV detector and using a C-18 column (length 250 mm, diameter 4.6 mm, particle size 5 µm) and a UV detector run at 215 nm. The limit of detection was 25 ± 3 µg/L. Three blank samples were obtained by spiking ultra-pure water with the studied herbicides to give final concentrations of 10 µg/L. Blanks were analysed as a control for memory effect in the instrument and for laboratory contamination. The average recoveries ranged from 83 to 90% for bentazone with relative standard deviations of 3-4%. All results were corrected with the corresponding recovery rates to provide accurate amounts.

Photo-degradation experiments under natural sunlight
The experiments were conducted using bentazone concentrations of 50 µM in filtered lagoon and canal water samples. The same concentrations in ultra-pure water were used for the estimation of the direct photolysis of bentazone. All experiments were conducted in triplicate. The samples were exposed to natural sunlight under summer conditions in Marseille (France). The samples were enclosed in 5 mL pyrex tubes (o.d. 1.3 cm, id. 1.1 cm). To simulate the environmental conditions, the tubes sealed with parafilm were placed in a basin filled with ultra-pure water which was wrapped with aluminium foil. The basin (diameter 30 cm, depth 15 cm) with the tubes was then placed on a roof at the laboratory in central Marseille. Starting on 9th of July 2008, the experiments were run for eight days. Aliquots of samples (200 µL) were withdrawn at various time intervals and were analysed by HPLC. Also, aqueous solutions of bentazone at 50 µM in ultra-pure water in tubes were wrapped in aluminium foil and kept in the dark to test for hydrolysis of the herbicide.

II. RESULTS AND DISCUSSION

Photodegradation under natural sunlight

The loss of bentazone was described as a sum of different first order kinetic processes as plots of (ln [bentazone]/[bentazone]$_o$) versus time and were found to be linear. The observable rate constant of herbicide degradation is defined as:

$k_{obs} = k_{dp} + k_{ip} + k_{hyd}$

which includes the first-order decay constant of direct k_{dp} (d^{-1}) and indirect photolysis k_{ip} (d^{-1}) and the first order decay constant of hydrolysis k_{hyd} (d^{-1}). The rate constants found for the Vaccarès lagoon water are given in Table 7.6/1.

Table 7.6/8 Summary of elimination rate constants for herbicides in the Vaccarès lagoon.

Elimination process	Elimination rate [d^{-1}]
Hydrolysis	k_{hyd} $0.001 \pm 1 \times 10^{-4}$
Direct photolysis	k_{dp} $0.14 \pm 2 \times 10^{-2}$
Indirect photolysis	k_{ip} $0.03 \pm 1 \times 10^{-2}$

The overall half-life times for bentazone were 2.17 ± 0.25 and 4.08 ± 0.32 d for canal and lagoon water samples, respectively. In ultra-pure water incubated in the dark, hydrolysis of bentazone was found to be negligible. The half-life time for bentazone in ultra-pure water under natural sunlight was measured to be 5.12 ± 0.7 d.

The authors admit that the light conditions for the photo-degradation experiments in glass vials differ from those naturally occurring in the lagoon which has an average depth of approximately 1 m and a natural turbidity. Using the model MASAS (Modelling of Anthropogenic Substances in Aquatic Systems) the overestimation of degradation rates under direct exposure was found to be by a factor of 2.5 to 3. The resulting photolytic half-life of bentazone under natural conditions would then be 12 d.

III. CONCLUSION

The authors concluded that direct and indirect photo-degradation of bentazone are main processes of dissipation in lagoon water. Half-lives of photo-degradation of bentazone were found to be 2.2 and 4.1 days under experimental conditions in canal and lagoon water, corresponding to an estimated half-life of 12 days in lagoon water in the field.

7.7 Ready biodegradability of the active substance

A study to test the ready biodegradability of bentazone was not performed. The substance has to be considered to be not readily biodegradable according to OECD guideline 301.

7.8 Degradation in aquatic systems

7.8.1 Aerobic biodegradation in aquatic systems

Since higher tier water/sediment studies were performed, no extra study on aerobic biodegradation is considered necessary.

7.8.2 Anaerobic biodegradation in aquatic systems

No EU requirement.

7.8.3 Water/sediment studies

The degradation of bentazone in water-sediment systems was evaluated during the last Annex I listing. No additional study was performed.

In a study with two aquatic systems ("Krempe" and "Ohlau"), most of bentazone remained in the water phase (53 - 61% of the total applied radioactivity (TAR) after 100 days). The maximum observed occurrence of bentazone in the sediment reached a maximum of 12.2% TAR at day 30 after treatment in system "Krempe". It slowly declined in sediment to 9.6% at 100 days.

N-methyl-bentazone was the only major metabolite found at a maximum of 12.5% TAR after 30 days in the water phase. It decreased again to about 2% after 100 days. N-methyl-bentazone is not expected to occur in significant amounts in sediments.

Mineralization was negligible with 2.6% TAR after 100 days. Formation of non-extractable residues was moderate reaching 13.4 - 15.6% TAR at the end of incubation.

The half-lives as listed in the EU review report (2000) are 161 days for the water phase and 523 and 908 days for the whole system.

Although the existing water/sediment study (*Bieber*, 1994, BASF DocID 1994/11026) is still considered valid, the guidance for the kinetic evaluation procedures has changed since last Annex I listing. Therefore, a new kinetic evaluation according to FOCUS degradation kinetics was performed. The old reported degradation rates are no longer valid.

Report:	II A 7.8.3/1 Matejek B. 2012(a) Kinetic evaluation of a water-sediment study with BAS 351 H - Bentazon according to FOCUS degradation kinetics BASF DocID 2011/1285046
Guidelines:	FOCUS (2006): Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration Sanco/10058/2005 version 2.0 434 pp.
Testing Laboratory and dates:	BASF SE; Limburgerhof; Germany Fed.Rep. 13-Jan-2012 - 13-Jan-2012
GLP:	No, not subject to GLP regulations

Executive Summary

The dissipation and degradation of BAS 351 H - bentazone in two water/sediment systems (Krempe and Ohlau) under aerobic dark conditions was evaluated according to the recommendations of the FOCUS workgroup on degradation kinetics.

The experimental data on bentazone in the test systems Krempe and Ohlau were evaluated at P-I level. The evaluation of the results of the Krempe test system at P-I level showed that degradation in the total system can be adequately described by SFO kinetics. Dissipation from the water phase was best described by the DFOP kinetic model, whereas dissipation from the sediment was best described by SFO kinetics. The evaluation of the results of the Ohlau test system at P-I level showed that degradation in the total system and dissipation from the water and sediment phase could be adequately described by SFO kinetics.

The estimated half-lives of bentazone in the two test system were: 207 and 283 days in the total system, 156 and 204 days in the water phase, and 171 and 179 days in the sediment phase.

P-II level analysis was performed for both systems but resulted in a weak fit to the measured data. Furthermore, for both systems t-test and F_{sed} check failed and thus kinetic evaluation at P-II level was not pursued further.

Metabolite evaluation at level M-I was not possible for any system. In the Krempe test system the identified metabolite N-methyl-bentazone was observed only at very low levels. The metabolite was found in a concentration > 5% AR only once: at day 30 (antepenultimate sampling point). All other samplings showed values clearly below 5% AR. In the Ohlau test system, N-methyl-bentazone reached a maximum concentration > 10% also at day 30 after application of the test substance. The compound was nearly exclusively found in the water phase with no appreciable detections in the sediment phase. All other samplings showed values clearly below 5% AR.

Furthermore, due to the late formation of the metabolite in both test systems only three time points each were available for kinetic fitting and no clear degradation pattern could be identified. Thus, the kinetic assessment of the metabolite did not provide any reliable results.

I. MATERIAL AND METHODS

Kinetic evaluation of the water/sediment study [*Bieber, W.-D. (1994): Degradation of the Test Substance Bentazon in Aerobic Aquatic Environment. - BASF DocID 94/11026*] was performed in order to derive persistence and modelling endpoints according to the current guidance of the FOCUS workgroup on degradation kinetics [*FOCUS (2006): "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp.*].
Experimental data of bentazone were analysed at the P-I level (one-compartment approach) for degradation in the whole system as well as dissipation from the water phase and dissipation in the sediment phase of the test systems. At the P-II level (two-compartment approach: water and sediment, see Box 10-2, p. 199 in *FOCUS (2006))*, the kinetic analysis considered the degradation in water and sediment and the partitioning between both phases.

Metabolite evaluation at level M-I was not possible for any system. In the Krempe test system the identified metabolite N-methyl-bentazone was observed only at very low levels. The metabolite was found in a concentration > 5% AR only once: at day 30 (antepenultimate sampling point). In the Ohlau test system, N-methyl-bentazone reached a maximum concentration > 10% also at day 30 after application of the test substance. The compound was nearly exclusively found in the water phase with no appreciable detections in the sediment phase.

However, due to the late formation of the metabolite in both test systems only three time points each were available for kinetic fitting and no clear degradation pattern could be identified. Thus, the kinetic assessment of the metabolite did not provide any reliable results.

Kinetic modelling

The appropriate kinetic model was identified considering the procedures and kinetic models proposed by the FOCUS workgroup on degradation kinetics [*FOCUS (2006)*, chapter 10]. The best-fit model was selected based on visual and statistical assessment and corresponding DT_{50} and DT_{90} values are reported as *persistence endpoints*. Appropriate DT_{50} values for use in environmental fate models were derived depending on the kinetic model and are reported as *modelling endpoints*.

Kinetic models included in the assessment

The kinetic models employed for this evaluation were described by the FOCUS workgroup on degradation kinetics.

Level P-I:

- Single-First-Order (SFO); Box 5-1, p. 51 in *FOCUS (2006)*,
- Gustafson and Holden (FOMC): Box 5-2, p. 53 in *FOCUS (2006)*,
- Hockey-stick (HS); Box 5-3, p. 55 in *FOCUS (2006)*,
- Double first-order in parallel (DFOP); Box 5-4, p. 57 in *FOCUS (2006)*.

Level P-II:

- Two-compartment model (water and sediment); Box 10-2, p. 199 in *FOCUS (2006)*.

Level M-I (dissipation):

- Single-First-Order (SFO); Box 5-1, p. 51 in *FOCUS (2006)*,
- Gustafson and Holden (FOMC): Box 5-2, p. 53 in *FOCUS (2006)*,
- Double first-order in parallel (DFOP); Box 5-4, p. 57 in *FOCUS (2006)*.

Level M-I (degradation plus formation):

- One-compartment model (parent and metabolite); Box 10-4, p. 224 in *FOCUS (2006)*.

Name and number of model parameters for the respective kinetic model fits are listed in *FOCUS (2006)*, Table 10-4 for parent compounds and Table 10-5 for metabolites.

The appropriateness of a distinct kinetic model to describe degradation can be tested with the following checks recommended by FOCUS [*FOCUS (2006)*, chapter 6.3.1]:

- Visual assessment of goodness-of-fit
- Estimation of the error percentage at which the χ^2 (chi²) test is passed (Equation 6-2; p. 89 in *FOCUS (2006)*)
- t-test to evaluate whether estimated degradation parameters differ from zero (Equation 6-3; p. 93 in *FOCUS (2006)*)

A kinetic model is considered appropriate if the residuals are randomly distributed, the χ^2 - error value is < 15 % and the estimated degradation parameters differ from zero as outlined by FOCUS [*FOCUS (2006)*, chapter 6.3.1 and chapter 10.2].

Data handling

Degradation in the whole system and dissipation from the water phase of the parent compound (P-I level) were evaluated starting on the day of treatment (i.e. 0 days after treatment, DAT 0). The dissipation of the parent from the sediment phase (P-I level) was evaluated starting at the day of maximum occurrence that was then defined as 0 days after maximum concentration (0 DAMC). All later times were adjusted accordingly as days after maximum concentrations (DAMC).

At P-I level of the analysis, the measured initial concentration of the parent substance in the total system or in water was set to the material balance recovered at DAT 0 as recommended by the FOCUS kinetics guidance. For the P-I analysis of sediment data, the maximum observed level of the parent compound in sediment was used as first data point for the evaluation.

At the P-II level, the total recovered amount at DAT 0 was considered as the measured initial concentration in water, while the initial concentration of the sediment phase was assumed to be zero.

At Level M-I, estimation of dissipation only requires kinetics to be fitted to the corresponding decline data for each compartment, starting from the maximum observed level of the metabolite in the compartment. The dissipation of the metabolite was thus evaluated starting at the day of maximum occurrence that was defined as 0 days after maximum concentration (0 DAMC). All later time points were adjusted accordingly as days after maximum concentrations (DAMC).

Estimating degradation in the whole water-sediment system at Level M-I requires fitting the whole system data to the kinetic model. Here, the total recovered amount at DAT 0 was considered as the measured initial concentration for the parent compound, while the initial concentration of the metabolite was assumed to be zero.

Values below the quantification or detection limit for parent compound and metabolites were treated as recommended by the FOCUS workgroup [*FOCUS (2006)*, chapter 6.1.4 and chapter 8.3.1].

Overview experimental data used for kinetic modelling

The experimental data used for kinetic analysis are shown in the following tables.

Table 7.8/1 Krempe test system: Experimental data of bentazone used for kinetic modelling

Amount of bentazone as [%] of TAR			
Days after treatment	**Whole System**	**Water**	**Sediment[#]**
0d/1	94.1[a]	94.1[b]	-[b]
0d/2	94.6[a]	94.6[b]	-[b]
0.25d/1	89.4	87.7	(1.7)[c]
0.25d/2	87.5	85.8	(1.7)[c]
1d/1	83.3	80.1	(3.2)[c]
1d/2	84.4	81.0	(3.4)[c]
2d/1	86.3	80.9	(5.4)[c]
2d/2	86.0	80.8	(5.2)[c]
7d/1	79.8	71.8	(8.0)[c]
7d/2	81.1	73.7	(7.4)[c]
14d/1	82.7	72.0	(10.7)[c]
14d/2	75.7	65.1	(10.6)[c]
30d/1	73.4	61.7	11.7
30d/2	77.4	65.8	11.6
60d/1	75.1	65.2	9.9
60d/2	72.9	62.6	10.3
100d/1	58.8	50.3	8.5
100d/2	65.1	55.8	9.3

[#] Calculated from study data as difference between whole system and water phase.
[a] The measured value at DAT 0 was set to the mass balance (total recovery).
[b] The measured value at DAT 0 was treated as if the substance was in the water phase.
[c] The data before the maximum occurrence in sediment were not considered for sediment phase modelling.

Table 7.8/2 Krempe test system: Experimental data for N-methyl-bentazone used for kinetic modelling

Amount of N-methyl-bentazone [%] as TAR			
Days after treatment	**Whole System**	**Water**	**Sediment[#]**
0d/1	-[a]	-[a]	-[a]
0d/2	-[a]	-[a]	-[a]
0.25d/1	(3.0)[b]	(3.0)[b]	0.0
0.25d/2	(2.4)[b]	(2.4)[b]	0.0
1d/1	(3.2)[b]	(3.2)[b]	0.0
1d/2	(2.7)[b]	(2.7)[b]	0.0
2d/1	(1.8)[b]	(1.8)[b]	0.0
2d/2	(1.5)[b]	(1.3)[b]	0.2
7d/1	(2.1)[b]	(2.1)[b]	0.0
7d/2	(1.9)[b]	(1.9)[b]	0.0
14d/1	(3.4)[b]	(3.4)[b]	0.0
14d/2	(3.1)[b]	(3.1)[b]	0.0
30d/1	8.8	8.8	0.0
30d/2	5.6	5.6	0.0
60d/1	1.0	1.0	0.0
60d/2	1.0	1.0	0.0
100d/1	4.7	4.5	0.2
100d/2	3.7	3.4	0.3

[#] Calculated from study data as difference between whole system and water phase.

[a] According to *FOCUS (2006)* metabolites which appear at time zero (t0) should be included as parent material. Hence, no t0 samples for the metabolite were considered in the assessment.

[b] The dissipation of the metabolite was evaluated starting at the day of maximum occurrence that was then defined as 0 days after maximum concentration (0 DAMC). All later times were adjusted accordingly as days after maximum concentrations (DAMC).

Table 7.8/3 Ohlau test system: Experimental data of bentazone used for kinetic modelling

Amount of bentazone [%] as TAR			
Days after treatment	**Whole System**	**Water**	**Sediment[#]**
0d/1	93.0[a]	93.0[b]	-[b]
0d/2	95.6[a]	95.6[b]	-[b]
0.25d/1	83.5	81.0	(2.5)[c]
0.25d/2	87.4	85.3	(2.1)[c]
1d/1	84.2	78.9	(5.3)[c]
1d/2	83.5	77.4	(6.1)[c]
2d/1	86.9	82.1	(4.8)[c]
2d/2	86.5	79.8	(6.7)[c]
7d/1	87.4	79.3	(8.1)[c]
7d/2	88.0	79.3	(8.7)[c]
14d/1	73.8	63.7	(10.1)[c]
14d/2	74.4	64.1	(10.3)[c]
30d/1	73.9	63.4	10.5
30d/2	61.7	52.2	9.5
60d/1	77.8	70.6	7.2
60d/2	80.5	71.7	8.8
100d/1	66.9	59.8	7.1
100d/2	70.1	62.0	8.1

[#] Calculated from study data as difference between whole system and water phase.
[a] The measured value at DAT 0 was set to the mass balance (total recovery).
[b] The measured value at DAT 0 was treated as if the substance was in the water phase.
[c] The data before the maximum occurrence in sediment were not considered for sediment phase modelling.

Table 7.8/4 Ohlau test system: Experimental data for N-methyl-bentazone used for kinetic modelling

Amount of N-methyl-bentazone [%] as TAR			
Days after treatment	**Whole System**	**Water**	**Sediment[#]**
0d/1	-[a]	-[a]	-[a]
0d/2	-[a]	-[a]	-[a]
0.25d/1	(2.2)[b]	2.2	0.0
0.25d/2	(2.4)[b]	2.3	0.1
1d/1	(2.2)[b]	2.0	0.2
1d/2	(1.8)[b]	1.8	0.0
2d/1	(1.3)[b]	1.0	0.3
2d/2	(0.4)[b]	(0.1)[b,c]	0.3
7d/1	(0.1)[b,c]	(0.1)[b,c]	0.0
7d/2	(0.1)[b,c]	(0.1)[b,c]	0.0
14d/1	(4.5)[b]	4.5	0.0
14d/2	(4.0)[b]	4.0	0.0
30d/1	10.0	10.0	0.0
30d/2	15.1	15.1	0.0
60d/1	1.6	1.6	0.0
60d/2	1.0	1.0	0.0
100d/1	2.4	2.4	0.0
100d/2	1.6	1.6	0.0

[#] Calculated from study data as difference between whole system and water phase.

[a] According to FOCUS (2006) metabolites which appear at time zero (t0) should be included as parent material. Hence, no t0 samples for the metabolite were considered in the assessment.

[b] The dissipation of the metabolite was evaluated starting at the day of maximum occurrence that was then defined as 0 days after maximum concentration (0 DAMC). All later times were adjusted accordingly as days after maximum concentrations (DAMC).

[c] Values <0.1 were set to 0.1.

Software for kinetic evaluation

The software package ModelMaker 3.0.4 [*Anonymous (1997) Model Maker User Manual, Version 3. Cherwell Scientific Publishing Limited.*] was used for parameter fitting and the kinetic models were defined as compartment models within the ModelMaker software. Non-linear regression methods (Marquardt algorithm, ordinary least squares optimization) as implemented in the Model Maker package were used. The optimization settings are described in the appendix of the original study report. The χ^2 - error levels and the reliability based on t-test were calculated using the Excel sheet FOCUS_DEGKIN_v2 provided by the FOCUS workgroup.

II. RESULTS AND DISCUSSION

The initial fit for all compartments (total system, water, sediment) was performed using SFO kinetics. In a further step, it was tested whether a bi-phasic FOMC kinetic model was more appropriate, and if so, DFOP and HS kinetics were implemented. Graphical presentations of the tested kinetic models and the results of the χ^2 - test and all other statistical endpoints used in the decision-making process are given in the original study report.

The degradation/dissipation kinetics of bentazone in the whole system, in the water phase and in the sediment phase was evaluated with regard to deriving persistence endpoints as well as modelling endpoints as described above.

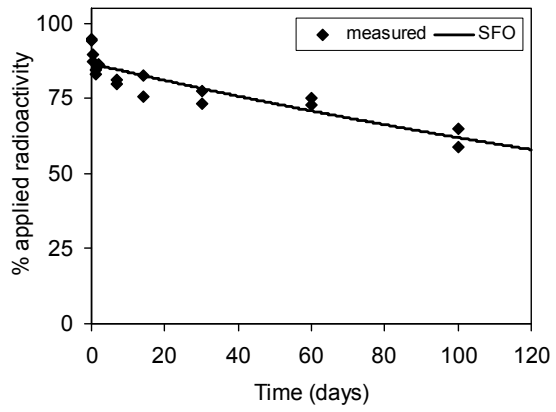
Summary of level P-I kinetic evaluation for Krempe system

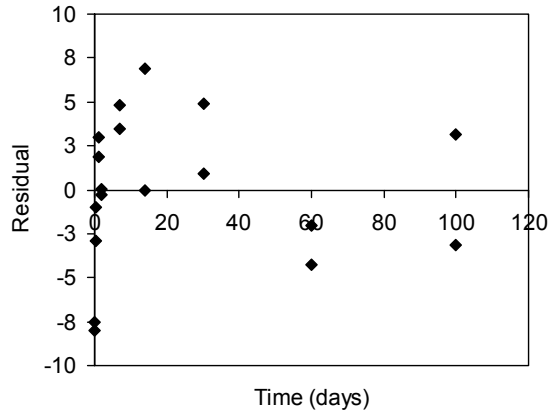
The evaluation of the results of the Krempe test system at P-I level showed that degradation in the total system was best described by SFO kinetics. Dissipation from the water phase was best described by the DFOP kinetic model, whereas dissipation from the sediment was best described by SFO kinetics.

The selected best-fit models are presented graphically in Figure 7.8/1, and a summary of the corresponding model parameters (including reliability based on t-test) is reported in Table 7.8/5.

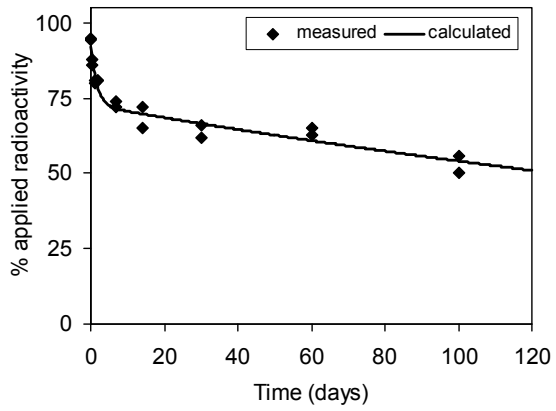
Figure 7.8/1 Level P-I, bentazone: best-fit kinetic models for total system, water and sediment (Krempe system)

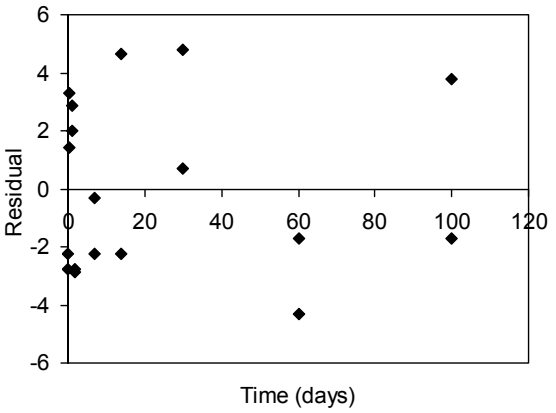
Total System – SFO kinetics



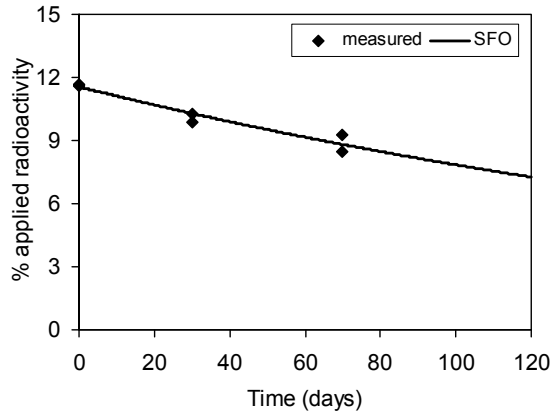


Water phase – DFOP kinetics





Sediment phase – SFO kinetics



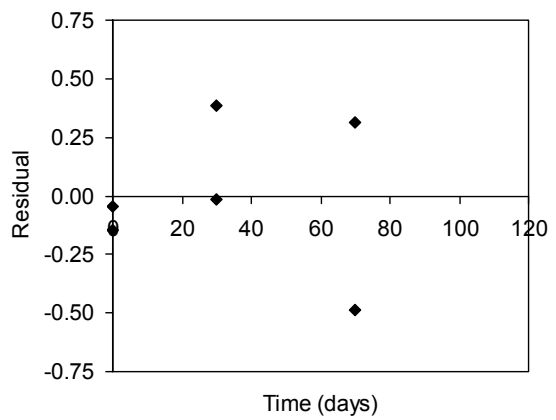


Table 7.8/5 Level P-I, bentazone: Estimated parameters for best-fit kinetic models (Krempe system)

Compartment	Kinetic model	Parameter	Estimated value	Standard error	Type I error rate	DT_{50} [d]	DT_{90} [d]
Total system*	SFO	M0 [%TAR]	86.593	1.281	-	207	688
		k [d^{-1}]	0.003	< 0.001	< 0.05		
Water**	DFOP	M0 [%TAR]	91.845	1.879	-	156	701
		k1 [d^{-1}]	0.600	0.200	< 0.05		
		k2 [d^{-1}]	0.003	0.001	< 0.05		
		g [-]	0.208	0.025	< 0.05		
Sediment**	SFO	M0 [%TAR]	11.555	0.230	-	179	595
		k [d^{-1}]	0.004	0.001	< 0.05		

* $DegT_{50}$
** $DisT_{50}$

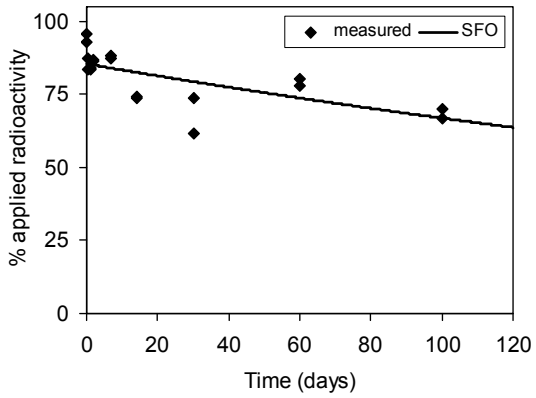
Summary of level P-I kinetic evaluation for Ohlau system

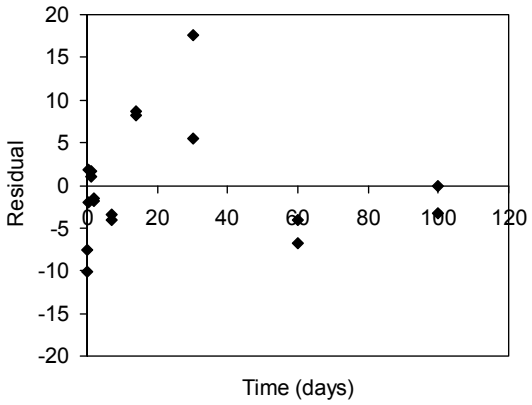
The evaluation of the results of the Ohlau test system at P-I level showed that degradation in the total system and dissipation from the water and sediment phase could be adequately described by SFO kinetics.

The selected best-fit models are presented graphically in Figure 7.8/2, and a summary of the corresponding model parameters (including reliability based on t-test) is reported in Table 7.8/6.

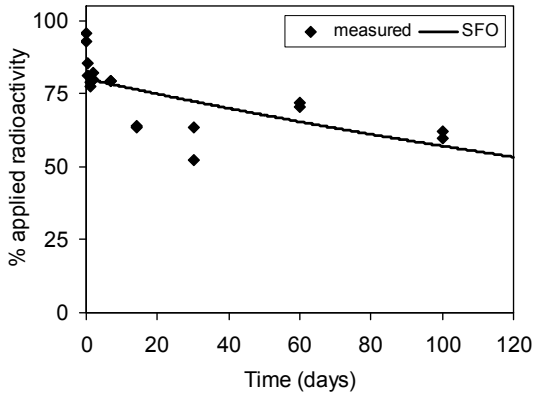
Figure 7.8/2 Level P-I, bentazone: best-fit kinetic models for total system, water and sediment (Ohlau system)

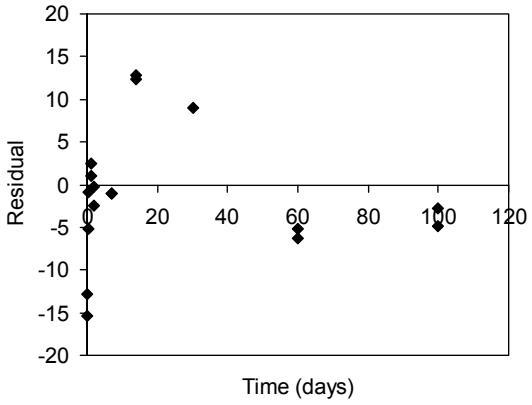
Total System – SFO kinetics



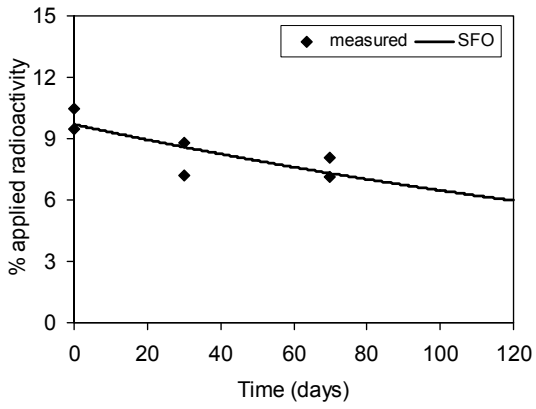


Water phase – DFOP kinetics





Sediment phase – SFO kinetics



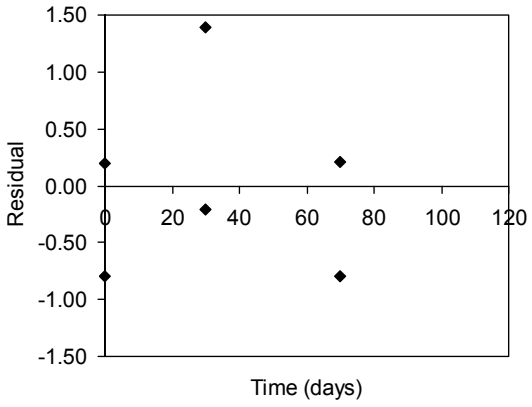


Table 7.8/6 Level P-I, bentazone: Estimated parameters for best-fit kinetic models (Ohlau system)

Compartment	Kinetic model	Parameter	Estimated value	Standard error	Type I error rate	DT_{50} [d]	DT_{90} [d]
Total system*	SFO	M0 [%TAR]	85.423	2.054	-	283	940
		k [d^{-1}]	0.002	< 0.001	< 0.05		
Water**	SFO	M0 [%TAR]	80.172	2.749	-	204	678
		k [d^{-1}]	0.003	< 0.00	< 0.05		
Sediment**	SFO	M0 [%TAR]	9.701	0.588	-	171	569
		k [d^{-1}]	0.004	0.002	< 0.05		

* $DegT_{50}$
** $DisT_{50}$

Summary of level P-II kinetic evaluation

Degradation of bentazone in water and sediment as well as partitioning between both phases was analysed according to the P-II level kinetic concept (two-compartment approach) of the FOCUS guidance document. A compartment model was used and SFO kinetics were considered for the transfer and degradation rates.

According to FOCUS [*FOCUS (2006)*, p. 203] it must be shown that the fit is visually and statistically acceptable, i.e. the results must be consistent with environmental fate data, degradation rates in both compartments must be significantly greater than zero as shown by t-test and the F_{sed} check must be passed.

P-II level analysis was performed for both systems but resulted in a weak fit to the measured data. For the Krempe system water phase, a visually acceptable fit could not be obtained, because the selected compartment model overestimated the degradation in the water phase. Also for the sediment phase, a visually acceptable fit could not be obtained. For the Ohlau system a visually acceptable fit could not be for either compartment.

Furthermore, t-test and F_{sed} check failed for both systems and thus kinetic evaluation at P-II level was not pursued further.

All graphical presentations of the kinetic models and the results of the χ^2 - test and all other statistical endpoints are shown in the original study report.

Summary of level M-I kinetic evaluation

Metabolite evaluation at level M-I was not possible for any system. In the Krempe test system the identified metabolite N-methyl-bentazone was observed only at very low levels. The metabolite was found in a concentration > 5% AR only once at day 30 (antepenultimate sampling point). All other samplings showed values clearly below 5 % AR see (Table 7.8/7).

In the Ohlau test system, N-methyl-bentazone reached a maximum concentration > 10% also at day 30 after application of the test substance. The compound was nearly exclusively found in the water phase with no appreciable detections in the sediment phase. All other samplings showed values clearly below 5 % AR (see Table 7.8/7).

Table 7.8/7 Maximum occurrence of N-methyl-bentazone in the two water-sediment systems

System	Replicate	Maximum occurrence		
		total system [%]	water [%]	sediment [%]
Krempe	1	8.8 (DAT 30) all other samplings < 5	8.8 (DAT 30) all other samplings < 5	0.2 (DAT 100)
	2	5.6 (DAT 30) all other samplings < 5	5.6 (DAT 30) all other samplings < 5	0.3 (DAT 100)
Ohlau	1	10.0 (DAT 30) all other samplings < 5	10.0 (DAT 30) all other samplings < 5	0.3 (DAT 2)
	2	15.1 (DAT 30) all other samplings < 5	15.1 (DAT 30) all other samplings < 5	0.3 (DAT 2)

However, due to the late formation of the metabolite in both test systems only three time points each were available for kinetic fitting and no clear degradation pattern could be identified. Thus, the kinetic assessment of the metabolite did not provide any reliable results.

III. CONCLUSION

The dissipation and degradation of bentazone in water/sediment systems under aerobic dark conditions was evaluated according to the recommendations of the FOCUS workgroup on degradation kinetics.

The experimental data on bentazone in the test systems Krempe and Ohlau were evaluated at P-I level.

The evaluation of the results of the Krempe test system at P-I level showed that degradation in the total system can be adequately described by SFO kinetics. Dissipation from the water phase was best described by the DFOP kinetic model, whereas dissipation from the sediment was best described by SFO kinetics. The evaluation of the results of the Ohlau test system at P-I level showed that degradation in the total system and dissipation from the water and sediment phase could be adequately described by SFO kinetics.

An overview of the estimated persistence endpoints of bentazone from the water/sediment study is given in Table 7.8/8. The table also shows the modelling endpoints that may be used for FOCUS surface water modelling at the EU level, derived according to FOCUS (Fig. 10-2, p. 198 in *FOCUS (2006)*).

P-II level analysis was performed for both systems but resulted in a weak fit to the measured data. Furthermore, for both systems t-test and F_{sed} check failed and thus kinetic evaluation at P-II level was not pursued further.

The metabolite N-methyl-bentazone was found in both water-sediment systems, but no reliable kinetic assessment could be conducted.

Table 7.8/8 Level P-I persistence and modelling endpoints for bentazone

Test System	Persistence endpoints			Modelling endpoints	
Total System	**Kinetic model**	**$DegT_{50}$ [d]**	**$DegT_{90}$ [d]**	**Kinetic model**	**$DegT_{50}$ [d]**
Krempe	SFO	207	688	SFO	207
Ohlau	SFO	283	940	SFO	283
				Geometric mean	**242**
Water phase	**Kinetic model**	**$DisT_{50}$ [d]**	**$DisT_{90}$ [d]**	**Kinetic model**	**$DisT_{50}$ [d]**
Krempe	DFOP	156	701	SFO[#]	235
Ohlau	SFO	204	678	SFO	204
				Geometric mean	**219**
Sediment phase	**Kinetic model**	**$DisT_{50}$ [d]**	**$DisT_{90}$ [d]**	**Kinetic model**	**$DisT_{50}$ [d]**
Krempe	SFO	179	595	SFO	179
Ohlau	SFO	171	568	SFO	171
				Geometric mean	**175**

[#] according to FOCUS (2006) the half-life from the slow phase of the DFOP bi-phasic model is used as modelling endpoint since 10% of the initially measured concentration was not reached within the experimental period

7.9 Degradation in the saturated zone

Information on the degradation behaviour of bentazone in deeper soil layers is given in chapter MII 7.2 *Rate of degradation in soil*.

7.10 Rate and route of degradation in air

Based on its physical-chemical properties, bentazone has no potential for volatilisation (4.9×10^{-6} Pa at 20°C). Furthermore, volatilisation from moist soil surfaces is not expected to be an important process for bentazone based on a Henry's law constant of 2.108 10 kPa m³/mol. Calculation of the atmospheric half-life shows that bentazone will be degraded by reaction with hydroxyl radicals with an estimated half-life of 2.1 hours. A long-range transport via air can therefore be excluded.

7.11 Definition of the residue

According to the results presented in chapters 7.1 - 7.10 the following compounds have to be considered for environmental risk assessments:

Soil	bentazone, N-methyl-bentazone
Groundwater	bentazone, N-methyl-bentazone
Surface water	bentazone, N-methyl-bentazone, peak B, peak C
Sediment	bentazone
Air	bentazone

N-methyl-bentazone (soil metabolite) was evaluated for its leaching risk as well as for its terrestrial and aquatic ecotoxicological potential. It proved to have no leaching risk and to have no unacceptable ecotoxicological effects.

Peak B and peak C (photo-degradates in aqueous photolysis) were evaluated on their potential aqua-toxicological effects. Both metabolites showed no risk of having harmful effects on aquatic organisms.

Therefore, bentazone is considered to be the only residue for potential monitoring in the environment.

7.12 Monitoring data concerning fate and behaviour

A large number of monitoring data of bentazone in surface water and groundwater are available in public literature or reports from agencies or authorities. Many of the monitoring studies are temporally and spatially limited or are related to a very specific aspect. They can therefore not considered representative for the overall situation of bentazone in surface and groundwater.

In this chapter a selection of those studies was included that were either carried out by BASF itself or which can be considered representative for the overall situation.

Open public literature that was found to address only very specific aspects or is restricted to locally very limited areas is considered not to be representative and was not included. Justification for the non-relevancy of each of these articles is given in the list of public literature “evaluated – not relevant” that was compiled from the literature search.

The first study summarized below was only included to show that storage stability of bentazone in water is proven for a period of more than 2 years, and that for low findings the possibility of insufficient storage stability can definitely be excluded.

Report:	II A 7.12/1 Grote C. 2006(b) Storage stability of BAS 351 H (Bentazone) in tap water (4°C) BASF DocID 2005/1031374
Guidelines:	EPA 171-4(e); IVA Guideline Residue Chemistry Part II Storage Stability 1992
Testing Laboratory and dates:	BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 07-Nov-2001 - 12-Dec-2003
GLP:	Yes (laboratory certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany Fed.Rep.)

Executive Summary

The storage stability of bentazone in tap water was investigated over a period of 25 months.

Tap water samples were spiked with the test item at a concentration level of 1.0 µg/kg. The spiked samples were stored in brown glass bottles in a refrigerator at a temperature of about + 4°C.

After 0, 1, 3, 6, 12, 24 and 25 months storage time, samples were analysed with BASF method 423 (GC/MS). The limit of quantitation of the method is 0.05 µg/kg. Procedural recoveries analysed within each analytical series proved the validity of the analytical method.

The analytical results used for the stability calculation were corrected for recoveries and were evaluated by means of an exponential function assuming that the degradation followed first order kinetics.

Within 25 months of storage, no decrease of bentazone could be observed. It can be concluded that water samples containing bentazone can be stored refrigerated (4°C) in the dark without significant loss of analytes at least over a period of 25 months.

I. MATERIAL AND METHODS

1. Test and reference item

BAS code:	BAS 351 H
Reg.No.:	51929
Chemical name (IUPAC):	3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide
Molecular weight:	240.28 g/mol (unlabelled)
Lot No.:	691-16-2 (PCP02718) (Reanalysis: PCP04954, PCP06668), supplied by BASF, APD/FC, Li 444
Purity:	99.8 %

2. Reference item N-methyl-bentazone

BAS code:	M351H009
Reg.No.:	79520
Chemical name (IUPAC):	3-isopropyl-1-methyl-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide
Molecular weight:	254.31 g/mol (unlabelled)
Lot No.:	L45-197 (PCP01057) (Reanalysis: PCP05500)
Purity:	99.7 %

3. Water

Tap water directly taken from the water pipe in Li 445 (Agricultural Center Limburgerhof) was used for analysis. It is supplied as a varying mixture obtained from three different wells.

A tap water sample taken from the pipe in Li 445 on Aug-23-2001 was characterized as given below.

Table 7.12/1 Characterisation of tap water

pH	7.81
Conductivity [µS/cm]	632
O_2 Content [mg/LI	7.7 (23.7°C [1])
Carbonate hardness [mmol/L]	5.39
Total hardness [mmol/L]	2.41
Total hardness [°dH] [2]	13.55
TOC (01/0505/01/1) (*) [mg/L]	2.2
DOC (01/0505101/1) (*) [mg/L]	2.6

[1] Related temperature
[2] Classification according to German hardness scale

A. STUDY DESIGN

1. Experimental Conditions

Nine 2.5 L amber glass bottles (one for each sampling interval, three as reserve) equipped with plastic screw caps and Teflon liner were pre-rinsed with tap water and then filled with 2.5 L tap water.

2.5 µg of bentazone dissolved in 1 mL methanol was added to each bottle resulting in a concentration of 1 µg/L. The test item solution was homogenized by shaking the bottles by hand. Afterwards, the glass bottles with the spiked water samples were placed in a refrigerator. The 0 day sample was analysed immediately after treatment. The storage samples were kept at a temperature of + 4°C ± 2°C in the dark over the entire period of the experiment.

2. Sampling

Samples were analysed after approximately 0, 1, 3, 6, 12, 24 and 25 month(s) of storage. The exact sampling dates are given in Table 7.12/2. Within each analytical set, at least one untreated tap water sample, two tap water samples freshly fortified at a concentration of 1.0 µg/kg (= procedural recovery samples) and two stored water samples are analysed.

3. Description of analytical procedures

All soil samples were analysed using BASF Analytical Method 423 [*Keller, W.: Validation of analytical method no. 423, determination of Bentazone residues in water BASF DocID 1998/10079*] with some modifications.

BASF method 423: Enrichment of bentazone from a 500 g acidified water sample by adsorption on a C18-SPE column. After desorption of the bentazone residues from the SPE column with acetone and the methylation with diazomethane, the formed N-methyl-bentazone is cleaned up by silica gel column chromatography. The final quantitative determination of N-methyl-bentazone is performed by GC/MS. The limit of quantitation is 0.05 µg/kg.

Modifications of BASF method 423
The three month storage samples could not be sucked through the extraction cartridge within 15 hours which most likely was caused by invisible particulate matter. Therefore, the remaining sample was extracted again with an additional cartridge but filled with a little amount of sea sand (approx. 1 cm height, Merck Germany, No.: 7712) and wool and conditioned the same way as the first ones. After derivatisation the extracts were combined before the analytical procedure continued with the column clean-up step as described in Keller, W., (1998).

All samples stored for > 3 months were first filtered through a folded paper filter (Macherey-Nagel, MN126/70 1/4, 24 cm diameter) and then extracted the same way as the three month storage samples. For blank and fortified samples the same modified cartridges were used. Elution of bentazone was performed with acetone (2 x 3 mL). During elution, the wool and sand were kept in the cartridge.

II. RESULTS AND DISCUSSION

Analysis of the untreated blank samples showed no significant matrix interferences. The results of the procedural recoveries, checked with freshly fortified samples, are listed in Table 7.12/2 together with the analytical results of the stored samples.

The storage samples were analysed in duplicate for bentazone residues. Uncorrected results as well as results corrected by the mean recovery are reported in Table 7.12/2. The recovery corrected residue data were used for the mathematical evaluation of the stability of bentazone.

The data in Table 7.12/2 demonstrate that bentazone is stable over the storage period of 25 months. The overall mean recovery was 93.2% with a relative standard deviation of 13.2% (n=14). These recovery values confirm the validity of the analytical method 423 used for this storage stability study.

Table 7.12/2 Analytical results: bentazone residues in stored water samples

Storage time [days]	Date of fortification	Date of analysis (extraction)	Procedural recovery [1] [%]	Mean proc. recovery [%]	Residues uncorrected [µg/kg]	Residues corrected [2] [µg/kg]
0	08.11.2001	08.11.2001	77.0	79.3	0.769	0.97
			81.6		0.824	1.04
28	08.11.2001	06.12.2001	80.8	81.1	0.846	1.04
			81.4		0.816	1.01
91	08.11.2001	07.02.2002	89.1	90.1	0.932	1.03
			91.1		0.937	1.04
179	08.11.2001	06.05.2002	96.8	93.9	1.00	1.06
			91.0		0.979	1.04
361	08.11.2001	04.11.2002	90.7	90.4	0.844	0.93
			90.1		0.827	0.91
732	08.11.2001	10.11.2003	98.4	103.7	1.13	1.09
			109.0		1.07	1.03
757	08.11.2001	05.12.2003	121.0	113.6	1.15	1.01
			106.1		1.12	0.99
		n	14			
		Mean	93.2			
		SD	12.3			
		%RSD	13.2			

[1] Freshly fortified at day of analysis; fortification level: 1.0 µg/kg

[2] Corrected with mean procedural recovery

III. CONCLUSION

The results of the study showed that water samples containing bentazone can be stored refrigerated (4°C) in the dark without significant loss of the analyte at least over a period of 25 months.

Surface water monitorings

Report:	II A 7.12/2 Laabs V. 2010(b) Surface water screening for Dicamba, Dimethenamide-P, Bentazone, Tritosulfuron, Topramezone and selected metabolites in three corn growing regions of the EU BASF DocID 2010/1148003
Guidelines:	
Testing Laboratory and dates:	BASF SE; Limburgerhof; Germany Fed.Rep. 14-Apr-2009 - 20-May-2010
GLP:	No

Executive Summary

The purpose of the study was the monitoring of five corn herbicides and their metabolites in selected surface water bodies in three corn growing regions of Europe. In the following, the results for bentazone are presented.

The Rott river (eastern Bavaria, Germany), the Adda and Oglio rivers (northern tributaries of the Po river, Italy) and the Sió and Danube river (central-western part of Hungary) were chosen, which all drain areas with relatively intensive cultivation of corn.

Surface water samples were taken bi-weekly during the application season and weekly thereafter for five months (April to beginning of September in Italy; May to beginning of October in Hungary), or weekly from Mai to November (Germany).

The analysis of samples was done centrally in the laboratory of BASF SE in Limburgerhof (Germany), using a multi-residue analysis method with a quantification limit of 0.01 µg/L for all analysed substances.

Bentazone was detected in surface water only at low concentrations with maximum peaks of 0.10 to 0.12 µg/L.

In general, the observed concentrations of bentazone in surface water are no cause for concern regarding ecotoxicological protection goals (as defined by environmental quality standards).

Furthermore, the measured concentrations are of no concern for drinking water production, as surface water is typically treated by oxidative and/or adsorptive measures before delivery to the consumer. By these routine procedures, the observed concentrations of bentazone in surface water can be reduced to levels <0.1 µg/L in finished drinking water.

I. MATERIALS AND METHODS

1. Test material

Reference substance

BAS code:	BAS 351 H
Common name:	Bentazone
Reg. No.:	51929
CAS-No.:	25057-89-0
Chemical name (IUPAC):	3-isopropyl-1H-2,1,3,-benzothiadiazin-4(3H)-one-2,2-dioxide
Chemical formula:	$C_{10}H_{12}N_2O_3S$
Molecular weight:	240.28
Purity:	99.8%
Lot. no.:	01893-210

2. Test sites

Sampling locations were chosen within regions of Europe where corn cultivation is one of the major cropping systems. The selection of monitoring points was in accordance with recommendations from national BASF and local independent scientific experts.

The Rott river (eastern Bavaria, Germany), the Adda and Oglio rivers (northern tributaries of the Po river, Italy) and the Sió and Danube river (central-western part of Hungary) were chosen, which all drain areas with relatively intensive cultivation of corn.

The surface water sampling spots were chosen to represent regionally independent catchments, providing information about the contamination situation in their basin areas.

An overview of the selected sites is given in Table 7.12/3.

Table 7.12/3 Sampling sites and sampling procedures

River	Rott (Germany)	Adda (Italy)	Oglio (Italy)	Sió (Hungary)	Danube (Hungary)
GPS coordinates	48.4282 N, 13.3341 E	45.1866° N, 9.7782° E	45.0415° N, 10.6499° E	46.3774° N, 18.7266° E	46.3522° N, 18.8945° E
Sample type	72-h time-integrated samples at several dates; grab samples (one sample) at all other sampling dates	Grab composite sample (three subsamples of river transect)			
Surrounding area	A mixture of cropland (corn, cereals) and scattered pastures dominates the land use in this region	Sampling spot located within the town centre of Pizzighettone (town surrounded by agricultural land)	Sampling spot located within agricultural land, dominated by cropped fields	Sampling spot surrounded by agricultural land cropped with corn and sunflowers	The larger area is dominated by agricultural land cropped with corn and sunflowers
Storm flow periods	Unknown	28 April, 01 May	28 April, 05 May	-	30 June, 07 July
Sampling device	Sampling tube of automated sampling device (72-h composite samples) or glass bottles	Horizontal water sampling bottle (PVC), inert silicone layer on inside		Horizontal water sampling bottle (PVC)	
Temperature	Unknown	12.4 to 23.5 °C	13.2 to 26.7 °C	16.4 to 27.6 °C	16.5 to 25.1 °C
pH	Unknown	7.9 to 8.9	8.0 to 8.9	8.2 to 9.1	7.5 to 9.4
Oxygen content	Unknown	7.0 to 8.1 mg/L	6.4 to 9.5 mg/L	62 to 250 % saturation	85 to 132 % saturation
Conductivity	Unknown	250 to 480 µS/cm	430 to 650 µS/cm	712 to 1145 µS/cm	292 to 416 µS/cm
Sample container	Glass bottles with Teflon-lined plastic screw caps	High-density polyethylene bottle (HDPE)			

4. Sampling and analysis

Surface water samples were taken biweekly during the application season and weekly thereafter for five months (April to beginning of September in Italy, May to end of September in Hungary), or weekly from May to November (Germany) in 2009.

At the sampling site Rott (Germany), sampling was done at a regular water monitoring site of the Bavarian Environmental Agency (LfU). The samples were either taken within the special pesticide monitoring program PSMRegio (72-h time-integrated samples, using an automated sampling device), or in between the regular intervals as grab samples using 1 L glass bottles. Samples were taken in ca. 30 cm depth, on the right-hand side of the river. Automated samples were pumped over a period of 72 h and cooled (<8°C) within the automated sampling device. During transport to the laboratory and storage samples were kept cool (<8°C).

At the sampling sites in Italy and Hungary, a horizontal water sampling bottle (cleaned, ethanol-rinsed, and once rinsed with river water before use) was submerged in the surface water body to ca. 30 cm depth below the water surface level for sampling. To receive a composite grab sample, three subsamples of ca. 1 L were taken from the cross-section of the rivers; subsamples were evenly spaced out to achieve a representative composite sample for the river at this point. The subsamples were unified and mixed, and a portion of the sample was used to measure basic water parameters on site (temperature, pH, conductivity, and oxygen saturation). The sampling bottles were closed and stored on (dry)ice immediately after sampling and stored deep frozen (<-16°C) until shipment on dry ice for analysis.

The analysis of samples was done centrally in the laboratory of BASF SE in Limburgerhof (Germany). Prior to routine sample analysis, an analytical multi-residue method was developed (BASF No. L149/01) and validated. For analysis, a 50 mL aliquot of the water sample was acidified with 6M HCl to pH 2 and concentrated on a solid phase extraction column; the column was then washed with purified water (pH 2). After drying the column for 1 minute under vacuum the residues were eluted with methanol. The eluate was reduced to dryness by evaporation and the residue was dissolved in methanol/water to prepare the final volume. The sample was finally measured using UPLC-MS/MS. The limit of quantification (LOQ) was determined at 0.010 µg/L, the limit of detection (LOD) at 0.002 µg/L.

5. Method validation

For quality control, fortified samples (minimum of two samples) were routinely analyzed with each batch of surface water samples. The recovery rates ranged from 67.5 % to 106.8 % of spiked amounts, with mean value of 85.6 %.

II. RESULTS AND DISCUSSION

Analysis results for bentazone are presented in Table 7.12/4.

In Germany, bentazone showed a maximum concentration of 0.10 µg/L in June. Bentazone could be detected in low amounts during the whole sampling period in samples of the Rott river. The substance concentrations measured in the 72-h time integrated samples fit well into the time course of concentrations from grab samples. Seemingly, pesticide peaks were of mid-term duration (several days) in the Rott river, while short-term peaks (e.g. from drift contamination) were not captured by the chosen sampling regime.

Samples of the two tributary rivers of the Italian Po river contained only low peak concentrations of bentazone (maximum 0.02 µg/L). Bentazone was found in traces or >LOQ during the whole sampling period in samples of the Oglio river. One possible explanation for the very low, but long-term quantifiable/detectable concentrations of bentazone in the Oglio/Adda rivers outside of the application period is a contribution of a long-term source (>3 months) to river water flow (e.g. recharge from groundwater).

In Hungarian surface water, measured peak concentrations were higher for the Sió river (0.01 to 0.12 µg/L) than for the Danube (<LOQ to 0.01 µg/L), as was to be expected due to dilution of pesticide peak concentrations in the bigger river. In both rivers, bentazone was detected in traces during most of the sampling period. In the Sió river, a transgression of the EU concentration limit of 0.1 µg/L for finished drinking water occurred only at one sampling event. If no drinking water is abstracted from the Sió river in this area without any treatment, the measured concentrations are of no immediate concern.

Table 7.12/4 Results of bentazone analysis at the five surface water sampling sites in three EU countries in 2009

Germany		Italy			Hungary		
Date	**Rott**	**Date**	**Adda**	**Oglio**	**Date**	**Sió**	**Danube**
6.5.09	<LOQ	**14.4.09**	-	<LOQ	**5.5.09**	0.02	<LOQ
***12.05.09**	0.02	**17.4.09**	-	<LOQ	**8.5.09**	0.02	<LOQ
18.5.09	0.02	**21.4.09**	-	<LOQ	**12.5.09**	0.01	<LOQ
***26.05.09**	0.06	**25.4.09**	-	<LOQ	**15.5.09**	0.01	<LOQ
***03.06.09**	0.10	**28.4.09**	-	<LOQ	**19.5.09**	0.03	0.01
10.6.09	0.10	**1.5.09**	-	<LOQ	**22.5.09**	0.05	<LOQ
17.6.09	0.04	**5.5.09**	-	<LOQ	**26.5.09**	0.05	<LOQ
***23.06.09**	0.02	**8.5.09**	<LOQ	<LOQ	**29.5.09**	0.02	<LOQ
30.6.09	0.02	**15.5.09**	-	<LOQ	**2.6.09**	0.06	<LOQ
7.7.09	0.01	**21.5.09**	-	<LOQ	**9.6.09**	0.06	<LOQ
***14.07.09**	0.01	**28.5.09**	-	0.01	**15.6.09**	0.02	<LOQ
22.7.09	<LOQ	**6.6.09**	<LOQ	<LOQ	**23.6.09**	0.02	<LOQ
28.7.09	<LOQ	**12.6.09**	-	0.01	**30.6.09**	0.09	0.01
10.8.09	<LOQ	**19.6.09**	<LOQ	0.02	**7.7.09**	0.12	0.01
19.8.09	0.01	**26.6.09**	<LOQ	0.01	**14.7.09**	0.04	<LOQ
26.8.09	0.01	**3.7.09**	<LOQ	0.01	**22.7.09**	0.03	<LOQ
1.9.09	0.01	**10.7.09**	<LOQ	<LOQ	**29.7.09**	0.02	<LOQ
8.9.09	0.01	**17.7.09**	<LOQ	0.01	**7.8.09**	0.01	<LOQ
15.9.09	0.01	**24.7.09**	<LOQ	0.02	**10.8.09**	0.01	<LOQ
***22.09.09**	0.01	**31.7.09**	<LOQ	0.02	**19.8.09**	0.01	<LOQ
5.10.09	0.01	**7.8.09**	<LOQ	0.02	**27.8.09**	0.02	<LOQ
***13.10.09**	0.01	**17.8.09**	<LOQ	0.01	**1.9.09**	0.02	<LOQ
21.10.09	0.01	**20.8.09**	<LOQ	0.01	**8.9.09**	0.01	<LOQ
29.10.09	<LOQ	**28.8.09**	-	<LOQ	**15.9.09**	0.05	-
***03.11.09**	<LOQ	**4.9.09**	<LOQ	<LOQ	**21.9.09**	0.03	-
		11.9.09	<LOQ	<LOQ	**29.9.09**	0.03	-

* Signifies 72-h time integrated sample

\- Signifies measured concentration <LOD

III. CONCLUSION

Bentazone was frequently detected in surface water in this study, but in quite low concentrations with maximum peaks (0.10 to 0.12 µg/L) barely transgressing even the EU drinking water limit of 0.1 µg/L.

In general, the observed concentrations of bentazone in surface water are no cause for concern regarding ecotoxicological protection goals (as defined by environmental quality standards) as well as drinking water production since water treatment procedure (oxidative and/or adsorptive measures) would reduce the concentration to levels <0.1 µg/L in finished drinking water.

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Report:	II A 7.12/3 Loos R. et al. 2008(a) Eu-wide survey of polar organic persistent pollutants in European river waters BASF DocID 2009/1127646
Guidelines:	None
Testing Laboratory and dates:	01-Sep-2008
GLP:	No, not subject to GLP regulations (scientific publication)

Executive Summary

This scientific publication describes investigations on the occurrence of polar organic persistent pollutants in European river waters. It was organized by the European Commission, Joint Research Centre, Institute for Environment and Sustainability (Ispra, Italy). Overall, 100 European rivers in 27 European countries were sampled and analysed for 35 selected organic compounds, comprising pharmaceuticals, pesticides, PFOS, PFOA, benzotriazoles, hormones and endocrine disruptors. Among the pesticides, bentazone was one of the analytes to be quantified in the water samples. The water sampling was performed in autumn which is considered to be outside the major application period for pesticides.

Overall, bentazone was found in 69% of the 122 samples. The highest single concentration found was 0.25 µg/L. The average concentration of bentazone was 0.014 µg/L and the median 0.004 µg/L. The 90^{th} percentile concentration was calculated to be 0.031 µg/L.

I. MATERIAL AND METHODS

Sampling and transport
Water sampling (122 samples in total) was done by overall 40 participating laboratories from 27 different EU Member States (research, public, as well as state-run laboratories). The JRC (Joint Research Center's Institute for Environment and Sustainability) gave advice to all the laboratories to perform the sampling preferably in the middle of the stream (from a bridge), or, when this was not possible, from the shore. Samples were to be collected about 30 cm below the water surface. Methanol pre-cleaned PE or PP plastic bottles (0.5 or 1 L) were provided to all laboratories and sampling teams. The participants were asked to fill these bottles, leaving a small air head-space, and to store them in a refrigerator at ~4 °C before dispatch by fast courier to Ispra (Italy). The samples were shipped cooled with freezing elements in styropor boxes and arrived generally after 2-3 days. Workup for analytics was started maximum 2 weeks after sampling.

Selection of sampling points
Since the sampling points in this study have been chosen upon proposal by the individual participating laboratories, many different water body types make part of this exercise, big and small, contaminated and pristine rivers and streams. The authors noted that big rivers and small streams are difficult to compare in terms of organic pollution. Small contaminated streams might not have a big relevance for the overall "environmental burden" for an ecosystem. For this reason, the participating labs were asked to provide also the individual mass flows of the rivers and streams in cubic meters per second (m^3/s), which ranged from ~2000 m^3/s for the Rivers Danube and Rhine, ~1500 m^3/s for the Rhone River, to very small streams with flows below 1 m^3/s. In total, 122 sampling stations from European streams and rivers were screened in this study.

Preparation of water samples
The water samples were extracted at the JRC Institute by solid-phase extraction (SPE) using Oasis HLB (200 mg) cartridges and an AutoTrace© SPE workstation. The extraction volume was 400 mL, and the water was not filtered. Before extraction, the samples were spiked with the internal standard, which contained the labelled substances PFOA $^{13}C_4$, PFOS $^{13}C_4$, carbamazepine db, simazine $^{13}C_3$, atrazine $^{13}C_3$, ibuprofen $^{13}C_3$, and 4n-nonylphenol d8. The spiking level in the water samples was 10 ng/L for PFOA $^{13}C_4$ and PFOS $^{13}C_4$, and 100 ng/L for the other labelled compounds. Elution was performed with 6 mL methanol.

Liquid chromatography tandem mass spectrometry (LC-MS/MS)
Analyses were performed by reversed-phase liquid chromatography (RP-LC) followed by electrospray ionization (ESI) mass spectrometry (MS) detection using atmospheric-pressure ionization (API) with a triple-quadrupole MS-MS system (Agilent 1100 Series LC coupled to a quattro micro MS from Waters-Micromass). Quantitative LC-MS analyses were performed in three separate LC-MS runs (method 1-3) in the multiple reaction monitoring (MRM) mode. Method 1 comprised the compounds in the negative ionization mode (among others bentazone), method 2 those in the positive ionization mode, and method 3 alkylphenolic compounds which were analysed with a different HPLC mobile phase.

Identification and quantification
The compounds were identified by retention time match and their specific LC-MS^2 MRM transitions. Good performance of the developed analytical methods was demonstrated by successful participation in several interlaboratory exercises on non-steroidal anti-inflammatory drugs (NSAIDs), PFAs and nonyl- and octylphenol. Quantification of the individual compounds was performed with similar internal standards (IS). For bentazone (as well as all other pesticides and pharmaceuticals), the internal standard ibuprofen $^{13}C_3$ was used.

The relative response factors of the compounds in relation to the IS were calculated in all cases. Thus, the reported concentrations are corrected with the recoveries of the compounds. A comparative check of internal quantification was always performed with external quantification. The limits of detection (LODs) for the SPE-LC-MS^2 procedure were calculated from the mean concentration of the blank of real water samples plus three times the standard deviation; 400 mL water was extracted and concentrated to 500 µL (enrichment factor 800). The measurement uncertainty is estimated to be around 25-50%. The LOD for bentazone was 1 ng/L.

Stability of the studied chemicals
The stability of the target compounds was investigated before the sampling campaign with a spiking experiment at 100 ng/L over a time period of 3 weeks using a real river water sample (River Olona, N-Italy). The spiked river water sample was stored in the laboratory outside the fridge at ~15 °C (in the dark). This experiment showed that bentazone (among others) were relatively stable over this time period, and from that the authors concluded that the risk of instability of the chemical during sample transportation at 4 °C was negligible.

II. RESULTS AND CONCLUSION

The average frequency of detection for all compounds was 61%. Pesticides were in general found in relatively low amounts. The authors assumed that this may be connected to the sampling time in autumn which is outside the major application period for most pesticides. The single highest pesticide concentration was found for isoproturon with 2 µg/L.

Bentazone was found in water samples at a frequency of 69% (LOD of 1 ng/L). The maximum bentazone concentration found was 0.25 µg/L. Within all samples it was found with an average concentration of 0.014 µg/L, a median concentration of 0.004 µg/L and a 90th percentile of 0.031 µg/L.

Groundwater monitorings

Executive Summary

I. MATERIAL AND METHODS

II. RESULTS AND DISCUSSION

III. CONCLUSION

Report:	II A 7.12/4 Bastiansen F. et al. 2009(c) Trend analysis of the results from groundwater monitoring of Bentazone in Germany BASF DocID 2009/1127662
Guidelines:	None
Testing Laboratory and dates:	BASF SE; Limburgerhof; Germany Fed.Rep. 30-Nov-2009 - 30-Nov-2009
GLP:	No, not subject to GLP regulations

Executive Summary

The groundwater monitoring data requested from the authorities of the federal states for the purposes of this study and received by BASF by the beginning of November 2009 are presented in this report together with a trend analysis.

The obtained data were included in a database and different analyses were conducted using several approaches for each of the federal states: Examinations on a yearly basis, examinations on a cumulative basis, examination of the current situation (2004-2008) and a trend analysis of the individual measuring points based on linear regression analysis.

For the individual federal states the results generally showed a positive trend. In comparison to the situation before 2004, the concentrations decreased in the majority of the cases; however, for some federal states only date since 2005 was available so that only the current situation could be described. The trend analyses of the individual measuring points showed a decreasing trend for the majority of the measuring points.
However, for a substantial amount of the measuring points no trend could be determined because there were either too few measurements for the respective measuring point or because no significant trend could be determined.

The following aspects were evident from the examination of all federal states together:

- A large number (almost two thirds) of the current findings which were relevant in the last 5 years were cases of “inherited pollution”. In some cases the original pollution event went back to considerably more than 5 years.
- About half of the groundwater measuring points (GWMP) with current limit value exceedances (LVE) in the last 5 years had fallen to below the limit value at the last sampling.
- On average, the number of GWMPs with LVEs at the last sampling made up less than 0.5% of all GWMPs investigated.
- The trend analysis for the individual measuring points showed a marked downward trend in the majority of cases. Evidence for an upward trend was found only in a very few isolated GWMPs.

Regarding the use of bentazone in sandy soils the evaluation showed that there was no difference between very sandy soils (n=985) and soils with lower sand content (n=12864) in the relative number of measuring points with limit value exceedances. This shows that sandy soils are not more vulnerable with regard to leaching of bentazone

I. MATERIAL AND METHODS

A. MATERIALS

Data was retrieved from different sources:

Data from existing BASF reports on bentazone

A series of clarification reports have already been submitted by BASF to the approval authorities for pesticide registration. In a large number of cases it was possible to identify reasons for groundwater contamination which showed no connection with leaching following application of bentazone-based products according to GAP. Although definite evidence could not be obtained in most of the other cases, it was possible to provide plausible indications that the most likely leaching pathway did not come about as a result of area-wide leaching after normal application of bentazone-based products.

Queries addressed to the federal states

The competent authorities of 14 federal states were asked to send an extract from their groundwater databases for the active substance bentazone, without any limitations or pre-selections with regard to the period of the examination or the results of analyses. To keep the queries within practicable limits, the information requested was confined to the following items: Number of the measuring point; name of the measuring point; coordinates (optional, subject to data protection regulations); municipality, rural district or urban district (possibly municipal code); sampling date; depth of filter(s) below ground level; concentration and unit used; data owner or data class; inclusion in agriculturally based measurement programmes; practical remarks on the measuring point.

Bentazone monitoring database

All individual readings presented in the BASF clarification reports and the data obtained from the federal states were imported into a BASF groundwater database and were finally included in the evaluations for this report.

The BASF monitoring database is in Microsoft Access format. The data administration software is GW-Base 7.0 from Ribeka. This software created the data base automatically and thus determined its internal structure. GW-Base 7.0 was also used for importing the measuring point master data and the readings. An overview of the available data in the readings database is shown in Table 7.12/5.

Table 7.12/5 Total available data in the readings database

FEDERAL STATE	START	END	NUMBER OF GW MEASUREMENT POINTS	NUMBER OF SAMPLES
Baden-Württemberg	1988	2009	3978	12050
Bavaria	1995	2009	2017	3682
Brandenburg	2000	2006	11	69
Hessen	1989	2009	4832	34248
Lower Saxony	1991	2009	47	48
North Rhine-Westphalia	1987	2009	2045	13066
Rhineland-Palatinate	1988	2009	25	45
Saarland	2005	2009	228	833
Saxony	1998	2006	109	644
Saxony-Anhalt	2003	2009	310	525
Schleswig-Holstein	1997	2009	42	129
Thuringia	1997	2008	312	1957
Total	**1987**	**2009**	**13956**	**67296**

B. STUDY DESIGN

The data was analysed using several approaches:

Examinations on a yearly basis
To analyse the temporal development of the number of groundwater measuring points (GWMPs) and the number of LVEs (limit value exceedance), the number of all GWMPs in any one federal state in which samples had been taken in a particular year was established. Also the number of GWMPs with LVEs in that year was worked out. To calculate the numbers of GWMPs with LVEs as percentage of the total number of GWMPs sampled, the number of GWMPs with LVEs were divided by the total number of GWMPs sampled:

$Percentage_{year}$ = GWMPs with $LVEs_{year}$ / total number of $LVEs_{year}$

A linear regression for the temporal development of the percentages of GWMPs with LVEs was performed for each federal state, using Microsoft Excel 2003.

Examinations on a cumulative basis
For this analysis for each year over the period of the analysis the GWMPs were identified which had been sampled for the first time in that year, and their number was recorded. Also for each year the GWMPs were identified which in that year had shown an LVE for the first time, and their number was recorded. The numbers of new GWMPs in a particular year or of new GWMPs with LVEs were then presented cumulatively.

Examination of the current situation
For this analysis, two time periods were analysed separately per federal state, i.e. the periods 1987-2003 and 2004-2008. In this way it was possible to assess the current situation using readings with a certain relevance to the present time, for which purpose a five-year period was chosen in view of the typical measuring programme cycles of the federal states. Thus the development over the last five years was compared with the situation in the preceding years. The numbers of GWMPs sampled and of GWMPs with LVEs were obtained for these periods from the database. Database queries were also carried out in order to calculate the numbers of GWMPs which showed LVEs during the first period, but in which no further LVEs were observed during the second period, despite continued sampling.

Trend analysis of the individual measuring points based on linear regression analysis
For the trend analysis of the individual measuring points, the readings for the GWMPs showing LVEs at any time during the period 1987-2008 were obtained from the database and used to evaluate the time series charts of the readings, leaving out the GWMPs which had been excluded from the analyses because of their close vicinity to neighbouring points; in those cases a common reason was assumed – based on the temporal dynamics of the concentrations and, if available, based on the entry path determined in the clarification reports. Only one of the measuring points was then included in the analysis. A linear regression of the time series charts of the concentrations was performed for each of the GWMPs using the R 2.6.2 software [R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3 900051-07-0, URL http://www.R-project.org].

All measurements from the first LVE onwards were used for the linear regression, since a level above the limit value for drinking water marks the beginning of the time period relevant for a trend analysis. Earlier historical readings without LVEs are without any relevance in this context. In cases where the first reading showing an LVE is at the same time the last known reading for a GWMP, the regression starts out from the first reading altogether for this GWMP.

II. RESULTS AND DISCUSSION

Results of the data analyses and statistics for Baden-Württemberg

Baden-Württemberg is the federal state with nationwide the largest number of GWMPs at which LVEs had at some time been measured, and also in which the largest number of GWMPs nationwide had ever been sampled. The trend analysis of Baden-Württemberg is thus an important indicator of the tendency generally to be expected in Germany.

In general the trend is positive. This is evident in particular from the fact that the bentazone concentrations of two thirds (66%; see Table 7.12/6) of the GWMPs with LVEs before 2004 had fallen below the limit value in the last 5 years. The fact that many of the GWMPs with LVEs had already been identified before 2004 shows that contamination in the majority of cases had taken place in the past.

A look at the GWMPs showing LVEs in the last 5 years shows that the concentrations at the final sampling had fallen below the limit value in more than half of the cases (56%; data not shown in table below), which points to a clearly decreasing trend in the number of LVEs. Thus the relative share of GWMPs with LVEs is at present not more than 0.4% of all GWMPs sampled (data not shown in table below).

The analysis of the concentration developments at the individual measuring points also shows a significant decrease in concentrations in the majority of all cases and serves to confirm the generally positive trend.

A summary of the analysis of the monitoring data from Baden-Württemberg is shown in Table 7.12/6.

Table 7.12/6 Summary of the monitoring data from Baden-Württemberg

Information on groundwater measuring points (GWMPs)	Number	Additional information
Overall period (1987-2008)		
GWMPs sampled from 1987-2008	3974	
GWMPs with at least one LVE in 1987-2008	85	
Period 1: 1987-2003 (without the last 5 years)		
GWMPs sampled in 1987-2003	2053	
GWMPs with at least one LVE in 1987-2003	67	79% of GWMPs with LVEs from overall period
Period 2: 2004-2008 (last 5 years)		
No more LVEs from GWMPs with LVEs in Period 1	49	
=> because levels had fallen below LV in 2004-2008	44	66% of GWMPs with LVEs from Period 1
=> because no measurements taken in 2004-2008	5	
Still LVEs from GWMPs with an LVE in Period 1	18	
GWMPs with first LVE in Period 2 (2004-2008)	18	
Total number of still relevant GWMPs (with LVEs in the last 5 years)	**36**	42% of GWMPs with LVEs from overall period
Trend in regression analysis		
Trend: Downward	17	47%
Trend: Upward	1	3%
Trend: not marked	9	25%
Too few measurements for a trend analysis	9	25%

Results of the data analyses and statistics for Hessen

Hessen, with almost 5000 GWMPs, is the federal state with nationwide the largest number of GWMPs investigated for bentazone. Hessen thus achieved the greatest area-related sampling density, and for this reason the situation in Hessen is of importance also for a supra-regional evaluation of bentazone occurrence in groundwater.

Bentazone findings above the limit value in groundwater occurred mostly before 1998 and by now are largely no longer relevant, since the concentrations in 43% of these cases can be shown to have fallen below the limit value (see Table 7.12/7). However no current data from the past 5 years are available for some of the GWMPs, which means that a further check would have to be carried out here to show whether these GWMPs also have fallen below the limit value.

In general it should be noted that, despite the large number of GWMPs sampled, the number of GWMPs with LVEs is very small. Whereas only 8 GWMPs showed an LVE during the last 5 years, this number was shown by the last current measurement to have sunk again by half, equivalent to an overall relative share of only 0.08% (data not shown in table below).

Moreover, all trends at the groundwater measuring points show that the concentrations have decreased. There is a significant decrease in the annual percentage of GWMPs with LVEs and also in the concentration developments for the individual measuring points, only very few of which (4) are currently above the limit value (data not shown in table below).

A summary of the analysis of the monitoring data from Hessen is shown in Table 7.12/7.

Table 7.12/7 Summary of the monitoring data from Hessen

Information on groundwater measuring points (GWMPs)	Number	Additional information
Overall period (1987-2008)		
GWMPs sampled from 1987-2008	4793	
GWMPs with at least one LVE in 1987-2008	31	
Period 1: 1987-2003 (without the last 5 years)		
GWMPs sampled in 1987-2003	4648	
GWMPs with at least one LVE in 1987-2003	23	74% of GWMPs with LVEs from overall period
Period 2: 2004-2008 (last 5 years)		
No more LVEs from GWMPs with LVEs in Period 1	23	
=> because levels had fallen below LV in 2004-2008	10	43% of GWMPs with LVEs from Period 1
=> because no measurements taken in 2004-2008	13	
Still LVEs from GWMPs with LVE in Period 1	0	
GWMPs with first LVE in Period 2 (2004-2008)	8	
Total number of still relevant GWMPs (with LVEs in the last 5 years)	**8**	26% of GWMPs with LVEs from overall period
Trend in regression analysis		
Trend: Downward	2	25%
Trend: Upward	0	0%
Trend: not marked	2	25%
Too few measurements for a trend analysis	4	50%

Results of the data analyses and statistics for North Rhine-Westphalia

North Rhine-Westphalia is the third federal state with both an extensive set of data and also a large number of measurements, some of which are considerably older than 5 years and thus suitable as a basis for a trend analysis.

The overall picture here confirmed the downward trend in the numbers of GWMPs with LVEs already shown by the evaluation of the data sets from Baden-Württemberg and Hessen. In this connection it should be emphasized that a substantial share (63%; see Table 7.12/8) of the GWMPs with concentrations above the limit value in Period 1 had been shown to have fallen to below the limit value over the last 5 years. It also emerged that, in much the same way as in Baden-Württemberg and Hessen, half of the GWMPs which had shown at least one LVE over the last 5 years were below the limit value at the last current sampling(data not shown in table below), which serves yet further to underline the downward tendency of the GWMPs with LVEs.

Finally, evidence for this downward trend can be found in the development of the numbers of GWMPs with LVEs as yearly percentages of the total number of GWMPs sampled – a development showing a statistically significant decrease with a 5% probability of error. Moreover, of the 7 GWMPs with LVEs over the last 5 years and for which a trend could be discerned, 6 showed a decrease in the concentration and only one showed an increase. A summary of the analysis of the monitoring data from North Rhine-Westphalia is shown in Table 7.12/8.

Table 7.12/8 Summary of the monitoring data from North Rhine-Westphalia

Information on groundwater measuring points (GWMPs)	Number	Additional information
Overall period (1987-2008)		
GWMPs sampled from 1987-2008	2032	
GWMPs with at least one LVE in 1987-2008	47	
Period 1: 1987-2003 (without the last 5 years)		
GWMPs sampled in 1987-2003	1464	
GWMPs with at least one LVE in 1987-2003	38	81% of GWMPs with LVEs from overall period
Period 2: 2004-2008 (last 5 years)		
No more LVEs from GWMPs with LVEs in Period 1	33	
=> because levels had fallen below LV in 2004-2008	24	63% of GWMPs with LVEs from Period 1
=> because no measurements taken in 2004-2008	9	
Still LVEs from GWMPs with LVE in Period 1	5	
GWMPs with first LVE in Period 2 (2004-2008)	9	
Total number of still relevant GWMPs (with LVEs in the last 5 years)	**14**	30% of GWMPs with LVEs from overall period
Trend in regression analysis		
Trend: Downward	6	43%
Trend: Upward	1	7%
Trend: not marked	4	29%
Too few measurements for a trend analysis	3	21%

Results of the data analyses and statistics for Thuringia

Since the total number of GWMPs sampled in Thuringia is smaller by a factor of 7-15 than in Baden-Württemberg, Hessen and North Rhine-Westphalia, the information value is much lower than in these other federal states.

A positive trend in the number of GWMPs with LVEs is discernible only for the last 5 years, and if this period is considered separately, the number of GWMPs with LVEs as a percentage of the total number of GWMPs sampled shows a significant decrease. However, a notably large number of the GWMPs with LVEs are in karst areas, which suggests that the limit value exceedances in most of the GWMPs with LVES are due to the greater vulnerability of karst areas.
A summary of the analysis of the monitoring data from Thuringia is shown in Table 7.12/9.

Table 7.12/9 Summary of the monitoring data from Thuringia

Information on groundwater measuring points (GWMPs)	Number	Additional information
Overall period (1987-2008)		
GWMPs sampled from 1987-2008	311	
GWMPs with at least one LVE in 1987-2008	15	
Period 1: 1987-2003 (without the last 5 years)		
GWMPs sampled in 1987-2003	184	
GWMPs with at least one LVE in 1987-2003	4	27% of GWMPs with LVEs from overall period
Period 2: 2004-2008 (last 5 years)		
No more LVEs from GWMPs with LVEs in Period 1	1	
=> because levels had fallen below LV in 2004-2008	1	25% of GWMPs with LVEs from Period 1
=> because no measurements taken in 2004-2008	0	
Still LVEs from GWMPs with LVE in Period 1	3	
GWMPs with first LVE in Period 2 (2004-2008)	11	
Total number of still relevant GWMPs (with LVEs in the last 5 years)	**14**	93% of GWMPs with LVEs from overall period
Trend in regression analysis		
Trend: Downward	4	29%
Trend: Upward	1	7%
Trend: not marked	6	43%
Too few measurements for a trend analysis	3	21%

Results of the data analyses and statistics for Bavaria

Bavaria, in addition to Baden-Württemberg, Hessen and North Rhine-Westphalia, is the fourth federal state for which an extensive set of data with results of bentazone monitoring are available. However, only data from the last 4 years were provided, so that it was not possible to work out a trend for GWMPs with LVEs over a longer period of time.

However, of the large number of GWMPs sampled for bentazone, only a small proportion (0.35%) still showed an LVE at the last sampling. There is also a discernible downward trend in the bentazone concentrations for the great majority of the GWMPs.

A summary of the analysis of the monitoring data from Bavaria is shown in Table 7.12/10.

Table 7.12/10 Summary of the relevant GWMPs with current LVEs (period 2005-2008) from Bavaria

Information on groundwater measuring points (GWMP)	Number	Additional information
GWMPs sampled from 2005-2008*	1989	
Total number of relevant GWMPs (with LVEs in the last 5 years)) 2005-2008*	12	
GWMPs in karst area	4-5	
GWMPs with direct contamination (>5 µg/L)	0	
GWMPs with clarification of findings	10	
Additional relevance considerations		
GWMPs below LV at last 3 samplings	1	8% of GWMPs with LVEs in the last 5 years
GWMPs below LV at final sampling	5	42% of GWMPs with LVEs in the last 5 years
GWMPs with LVEs at final sampling	**7**	58% of the total number of GWMPs with LVEs (2005-2008*)
		0.35% of the total number of GWMPs sampled (1987-2008)
Trend in regression analysis		
Trend: Downward	5	42%
Trend: Upward	0	0%
Trend: not marked	5	42%
Too few measurements for a trend analysis	2	17%

* No data earlier than 2005 received

Results of the data analyses and statistics for Saxony-Anhalt

The total number of GWMPs sampled in Saxony-Anhalt is smaller by a factor of 7-15 than in Baden-Württemberg, Hessen, North Rhine-Westphalia and Bavaria. Hence the information value of the data from Saxony-Anhalt is much lower than from the other federal states referred to.

A positive trend in the development of the GWMPs with LVEs can be discerned from the fact that more than a quarter of the GWMPs showing LVEs over the course of the observation period were below the limit value at the last sampling.

It should be noted that a considerable proportion of the GWMPs with LVEs are located in karst areas. The greater vulnerability of these areas is probably the cause of the LVEs. Many of the critical sites are in the vicinity of rivers, which means it will be necessary to investigate the influence of surface water infiltration on the GWMPs with LVEs.
A summary of the analysis of the monitoring data from Saxony-Anhalt is shown in Table 7.12/11.

Table 7.12/11 Summary of the relevant GWMPs with current LVEs (period 2005-2008) from Saxony-Anhalt

Information on groundwater measuring points (GWMPs)	Number	Additional information
GWMPs sampled from 2005-2008*	309	
Total number of relevant GWMPs (with LVEs in the last 5 years)) 2005-2008*	15	
GWMPs in karst area	2-5	
GWMPs with direct contamination (>5 µg/L)	3	
GWMPs with clarification of findings	3	
Additional relevance considerations		
GWMPs below LV at last 3 samplings	0	0% of GWMPs with LVEs in the last 5 years
GWMPs below LV at final sampling	4	27% of GWMPs with LVEs in the last 5 years
GWMPs with LVEs at final sampling	**11**	73% of the total number of GWMPs with LVEs (2005-2008*)
		3.56% of the total number of GWMPs with LVEs (1987-2008)
Trend in regression analysis		
Trend: Downward	1	7%
Trend: Upward	0	0%
Trend: not marked	4	27%
Too few measurements for a trend analysis	10	67%

* No data earlier than 2005 received

Results of the data analyses and statistics for Saarland

The total number of GWMPs sampled in Saarland is smaller by a factor of more than 10-20 lower than in the federal states with the largest numbers of samplings. All the same, the sampling density is relatively high in relation to the farmland area. Because of the small number of GWMPs with LVEs and the fact that all of the LVEs were measured in only one year, a trend analysis cannot be carried out. However, at the one measuring point for which several measurements were available it was possible to identify a statistically significant downward trend.

A summary of the analysis of the monitoring data from Saarland is shown in Table 7.12/12.

Table 7.12/12 Summary of the relevant GWMPs with current LVEs (period 2005-2008) from Saarland

Information on groundwater measuring points (GWMPs)	Number	Additional information
GWMPs sampled from 2005-2008*	222	
Total number of relevant GWMPs (with LVEs in the last 5 years)) 2005-2008*	3	
GWMPs in karst area	0	
GWMPs with direct contamination (>5 µg/L)	0	
GWMPs with clarification of findings	0	
Additional relevance considerations		
GWMPs below LV at last 3 samplings	0	0% of GWMPs with LVEs in the last 5 years
GWMPs below LV at final sampling	1	33% of GWMPs with LVEs in the last 5 years
GWMPs with LVEs at final sampling	**2**	67% of the total number of GWMPs with LVEs (2005-2008*)
		0.90% of the total number of GWMPs with LVEs (2005-2008)
Trend in regression analysis		
Trend: Downward	1	33%
Trend: Upward	0	0%
Trend: not marked	0	0%
Too few measurements for a trend analysis	2	67%

* No data earlier than 2005 received

Results of the data analyses and statistics for Saxony

The number of GWMPs available for Saxony is much smaller than for all other federal states, since use could be made here only of data for selected rural districts.

It was found that all GWMPs which had already shown LVEs in Period 1 (up to 2003) were to some extent cases of “inherited pollution”, though 60% of them had shown no more LVEs in the last 5 years.

One of the two remaining GWMPs with LVEs in the last 5 years showed a downward trend. The concentrations decreased at the last 3 measurements to below the limit value.

A summary of the analysis of the monitoring data from Saxony is shown in Table 7.12/13.

Table 7.12/13 Summary of the monitoring data from Saxony

Information on groundwater measuring points (GWMPs)	Number	Additional information
Overall period (1987-2006)		
GWMPs sampled from 1987-2006	109	
GWMPs with at least one LVE in 1987-2006	5	
Period 1: 1987-2003 (without the last 5 years)		
GWMPs sampled in 1987-2003	84	
GWMPs with at least one LVE in 1987-2003	5	100% of GWMPs with LVEs from overall period
Period 2: 2004-2008 (last 5 years)		
No more LVEs from GWMPs with LVEs in Period 1	3	
=> because levels had fallen below LV in 2004-2008	3	60% of GWMPs with LVEs from Period 1
=> because no measurements taken in 2004-2008	0	
Still LVEs from GWMPs with LVE in Period 1	2	
GWMPs with first LVE in Period 2 (2004-2008)	0	
Total number of still relevant GWMPs (with LVEs in the last 5 years)	**2**	40% of GWMPs with LVEs from overall period
Trend in regression analysis		
Trend: Downward	1	50%
Trend: Upward	0	0%
Trend: not marked	1	50%
Too few measurements for a trend analysis	0	0%

Results of the data analyses and statistics for all federal states from which data were made available

In the following a summary of all eight federal states (Baden-Württemberg, Hessen, North Rhine-Westphalia, Thuringia, Bavaria, Saxony-Anhalt, Saarland and Saxony) is shown. However, it should be mentioned that the account is somewhat distorted by the fact that the data made available by some of these states were confined to Period 2 (2004-2008).

A total of 13774 GWMPs were sampled in these 8 states, and in 213 of these at least one LVE was observed at some time or other over the entire sampling period. Just over 60% of the total number of GWMPs sampled had already been sampled during Period 1 (1987-2003). This means that over the last 5 years the measurement network of the states had been extended by almost 40%.

About two thirds of the GWMPs with LVEs were cases of “inherited pollution”, with a history going back more than 5 years. In 60% of these no further exceedances had been measured in the last 5 years. During these last 5 years a total of 104 GWMPs with LVEs were reported.

Table 7.12/14 Summary of the monitoring data from all federal states included in this study

Information on groundwater measuring points (GWMPs)	Number	Additional information
GWMPs sampled from 1987-2008	13774	
GWMPs with at least one LVE in 1987-2008	213	
Period 1: 1987-2003 (without the last 5 years)		
GWMPs sampled in 1987-2003	8433	
GWMPs with at least one LVE in 1987-2003	139	65% of GWMPs with LVEs from overall period
Period 2: 2004-2008 (last 5 years)		
No more LVEs from GWMPs with LVEs in Period 1	109	
=> because levels had fallen below LV in 2004-2008	82	59% of GWMPs with LVEs from Period 1
=> because no measurements taken in 2004-2008	27	
Still LVEs from GWMPs with LVE in Period 1	26	
GWMPs with first LVE in Period 2 (2004-2008)	49	
Total number of still relevant GWMPs (with LVEs in the last 5 years)	**104**	49% of GWMPs with LVEs from overall period
Trend in regression analysis		
Trend: Downward	37	36%
Trend: Upward	3	3%
Trend: not marked	31	30%
Too few measurements for a trend analysis	33	32%

As far as current relevance is concerned, the concentration measured at the last sampling had fallen to below the limit value in almost half of all cases, so that the GWMPs with LVEs made up 0.41% of all GWMPs analysed for bentazone during the overall period.

Table 7.12/15 Summary of the relevant GWMPs with current LVEs (period 2004-2008) from all federal states included in this study

Information on groundwater measuring points (GWMPs)	Number	Additional information
Total number of still relevant GWMPs (with LVEs in the last 5 years) 2004-2008	104	
GWMPs in karst area	24-29	
GWMPs with direct contamination (>5 µg/L)	4	
GWMPs with clarification of findings	55	
Additional relevance considerations		
GWMPs below LV at last 3 samplings	15	14% of GWMPs with LVEs in the last 5 years
GWMPs below LV at final sampling	48	46% of GWMPs with LVEs in the last 5 years
GWMPs with LVEs at final sampling	**56**	54% of GWMPs with LVEs in the last 5 years
		26% of the total number of GWMPs with LVEs (1987-2008)
		0.41% of the total number of GWMPs sampled (1987-2008)

The summary of the trend analyses for the individual measuring points shows that there is a discernible downward trend in over one third (and hence in the majority) of all GWMPs which had an LVE in the last 5 years. An increase was discernible in only a few isolated cases. The trend in another third was not marked, and in the remaining third there were too few measurements for a trend analysis.

Table 7.12/16 Trend analysis of the relevant GWMPs with current LVEs (period 2004-2008) from all federal states included in this study

Information on groundwater measuring points (GWMPs)	Number	Additional information
Total number of still relevant GWMPs (with LVEs in the last 5 years) 2004-2008	104	
Trend: downward	37	36%
Trend: upward	3	3%
Trend not marked	31	30%
Too few measurements for a trend analysis	33	32%

Discussion and conclusions from the trend analysis of the monitoring results from all federal states

The following aspects were evident also from the examination of all federal states together:

- A large number (almost two thirds) of the current findings which were relevant in the last 5 years were cases of “inherited pollution”. In some cases the original pollution event went back to considerably more than 5 years.
- About half of the GWMPs with current LVEs in the last 5 years had fallen to below the limit value at the last sampling.
- On average, the number of GWMPs with LVEs at the last sampling made up less than 0.5% of all GWMPs investigated.
- The trend analysis for the individual measuring points showed a marked downward trend in the majority of cases. Evidence for an upward trend was found only in a very few isolated GWMPs.

Evaluation of the available monitoring data on findings in areas with very sandy soils

To explore the extent to which the LVEs might be caused by leaching of bentazone in locations with very sandy soils of the types pure sand, slightly silty sand and slightly clayey sand, an analysis was carried out for all GWMPs with LVEs in the monitoring database. This was done to show how many of them were located in areas with very sandy soils.

The present analysis takes into account all GWMPs in the database and from all federal states from which data were available. This was done regardless of whether comprehensive monitoring data had been received from the federal states in question, in contrast to the examinations in the previous chapters, where this was required. Thus this analysis includes all GWMPs which had shown at least one LVE since 1987.

A summary of the data on GWMPs sampled for bentazone and also on GWMPs with LVEs in areas with very sandy and less sandy soils is shown in Table 7.12/17.

It was found that the numbers of GWMPs with LVEs from areas with very sandy soils formed the same percentage of the total number of GWMPs sampled as from areas with less sandy soils. From this it can be concluded that sandy soils are not more vulnerable with regard to leaching of bentazone. The large relative share of almost 1000 GWMPs in very sandy areas and almost 13000 GWMPs in non-sandy areas can be taken as representative and reliable statistic evidence.

The relatively high percentages (> 1%) came about because the examinations comprised all GWMPs which had ever shown an LVE at any time, even if these included cases of “inherited pollution” (some them going back a long time) and GWMPs which had shown no further LVEs for many years. If the examination had been confined to GWMPs with relevant current LVEs from the last 5 years, this share would have been considerably less than one percent.

Table 7.12/17 Breakdown of GWMPs with and without LVEs in areas with very sandy and less sandy soils, for all federal states for which data are contained in the monitoring database

Federal state	GWMPs in very sandy soils	GWMPs in less sandy soils	GWMPs with LVEs in very sandy soils	GWMPs with LVEs in less sandy soils	Percentage of GWMPs with LVEs in very sandy soils	Percentage of GWMPs with LVEs in less sandy soils
Baden-Württ.	1	3954	1	84	100.0%	2.1%
Bavaria	62	1934	1	11	1.6%	0.6%
Hessen	480	4335	2	29	0.4%	0.7%
North Rhine-Westphalia	287	1745	4	43	1.4%	2.5%
Schleswig-Holstein	13	28	1	6	7.7%	21.4%
Saxony- Anhalt	54	255	1	14	1.9%	5.5%
Lower Saxony	18	14	3	5	16.7%	35.7%
Saxony	63	46	1	4	1.6%	8.7%
Brandenburg	2	9	0	1	0.0%	11.1%
Rhineland-Palatinate	5	11	3	3	60.0%	27.3%
Saarland	0	222	0	3	-	1.4%
Thuringia	0	311	0	15	-	4.8%
Total	**985**	**12864**	**17**	**218**	**1.7%**	**1.7%**

III. CONCLUSION

Products containing bentazone have been registered for use in the Federal Republic of Germany since 1972. As regards leaching into groundwater, the introduction in 1991 of the limit value for drinking water of 0.1 µg/L for the individual active substance as a criterion for authorization resulted accordingly in a reduction of the maximum permissible application rate to 1 kg active substance per hectare for all products containing bentazone. This means that for more than 15 years there was an authorization for application rates which from 1991 onwards were classified as too high in terms of a possible hazard to groundwater. The first available data from the groundwater monitoring databases of the federal states from the late 1980s and early 1990s already showed signs of contamination for this period.

At the same time, the detection of crop protection products in groundwater and the inclusion of this issue in professional training have helped to bring about a growing awareness among farmers that their individual practice can contribute greatly to the avoidance of environmental contamination. Bentazone is an active substance which binds only to a limited extent to soil and therefore, in the event of faulty application, has fewer intrinsic safety reserves as regards groundwater contamination. The described higher application rates before 1991 together with farming practices which resulted locally in excessive concentrations in soil or water (e.g. handling of leftover product) led to groundwater contaminations over 37 years of application which can also be described as inherited pollution. How long it will take before an inherited contamination of this kind is detected in a groundwater measuring point, a spring or an extraction well depends greatly on the hydrogeological situation at the original point of contamination and the distance from the measuring point. As a rule, the age of the groundwater can be used as an orientation value. In most cases no measured values are available for this purpose, but it can range in more shallow groundwater from a few years to several decades.

The review and trend analysis given in this report on the basis of data from 8 federal states which had been collected between 1987 and 2008 present the whole of the data currently available on the condition of groundwater. Despite the difficulties in interpreting the data due to the constant changes in sampling coverage, an analysis of the limit value exceedances in relation to all measured values available revealed that there was a more or less clearly marked downward trend in Baden-Württemberg, Hessen and North Rhine-Westphalia. An analysis of the bentazone concentration developments at each of the measuring points in question showed that there were very many more measuring points with a downward than with an upward trend. However, there were a noteworthy number of measuring points at which it was not yet possible to discern any trend, and there are still measuring points at which bentazone was detected for the first time. However, this is inevitable wherever inherited contamination is present, due to the movement of the groundwater.

Without reference to the evaluation of the trends, BASF carried out a clarification of findings for all limit value exceedances in groundwater known at the time in question. This was done in order to study the connection with leaching following correct application of bentazone-based products and to deduce the most probable cause of contamination. Apart from direct contamination into defective wells or measuring points, inflow of waste water, farmyard run-off, etc. it turned out that a large proportion of the limit value exceedances were to be found in the hydrological catchment areas of surface waters. There is also reason to suppose a connection with karst structures, though in many cases this went together with the influence of surface water or waste water on the measuring point concerned.

Management measures

The use requirements referred to in the Introduction were issued by the Federal Office for Consumer Protection and Food Safety (BVL) in 2005. As far as the restriction on use in sandy soils is concerned, the evaluation in this report showed that there was no difference between very sandy soils (n=985) and soils with lower sand content (n=12864) in the relative number of measuring points with limit value exceedances. With reference to the ban on use in very sandy soils, which first came into effect in the 2005, 2006 and 2007 seasons, it is too early to expect any influence on the groundwater concentrations evaluated up to 2008. On the basis of the monitoring data, therefore, there is no higher leaching potential in connection with very sandy soils.

The present review of bentazone in groundwater brought to light a number of previously unknown discovery sites, for which further clarifications of the findings must be carried out.

In this context, the concrete indications of the influence of karst structures and surface waters should be subject to further investigations and if necessary find a reflection in use requirements.

Report:	II A 7.12/5 Dressel J., Grote C. 2002(b) Monitoring study on residues of BAS 351 H (Bentazone) in deep groundwater beneath treated areas in the Netherlands BASF DocID 2002/1005310
Guidelines:	EEC 96/46; Guidance Document on Residue Analytical Methods (SANCO/825/00rev.6); EEC 91/414 Annex IIA; EEC 91/414 Annex IIIA; Handleiding Toelating Bestrijdingsmiddelen - Risico voor milieu - Gebruik meetgegevens
Testing Laboratory and dates:	BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. 15-Oct-2001 - 30-Apr-2002
GLP:	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

A groundwater monitoring study was conducted to assess the leaching risk for Dutch groundwater after use of bentazone according to the current label and good agricultural practice. 108 wells of the national and provincial groundwater monitoring networks were selected for an effective depth range from 6 m to 14 m, land use and travel time of the sampled water from the soil surface. Sampling, sample transport and chemical analysis were conducted according to the principles of Good Laboratory Practice (GLP). 102 filters were sampled once whereas 6 filters were not sampled due to difficulties in finding the wells. The information gathered during the sampling led to a further de-selection of 10 well filters which showed irregularities. The remaining 92 data points show 4 values above 0.1 µg/L and a 90th percentile of 0.025 µg/L which happens to be the lowest value possible as all samples with concentrations below the limit of quantitation (LOQ) were assigned half of the LOQ (=0.025 µg/L) for this assessment.

Therefore more than 90 % of the groundwater samples taken throughout the Netherlands showed concentrations of less than 0.1 µg/L, and it can be concluded that the use of bentazone poses no risk for the contamination of groundwater in 10 m depth in the Netherlands.

I. MATERIALS AND METHODS

1. Test material

Reference substance used for fortifications

BAS code:	BAS 351 H
Common name:	Bentazone
Reg. No.:	51929
CAS-No.:	25057-89-0
Chemical name (IUPAC):	3-isopropyl-1H-2,1,3,-benzothiadiazin-4(3H)-one-2,2-dioxide
Chemical formula:	$C_{10}H_{12}N_2O_3S$
Molecular weight:	240.28
Purity:	99.8%, homogeneous
Lot. no.:	691-16-2 (PCP02718) (Date of reanalysis: 27.07.1998; PCP04954)
Stability:	Expected to be stable at least for 5 years when stored at room temperature

2. Test sites and monitoring wells

108 wells of the national and provincial Dutch groundwater monitoring networks were selected based on their effective soil depth range from 6 m to 14 m (maximum range for filter position), land use and travel time of the sampled water from the soil surface. 102 filters were sampled once whereas six filters were not sampled due to difficulties in finding the wells. The selection of wells was performed by evaluating the well characteristics, as provided by RIVM (Dutch National Institute for Public Health and the Environment). Criteria were as follows:

- sampling depth in the well, based on the filter position, was 6 to 14 m below the soil surface, providing a range that was equally distributed around the target depth of 10 m
- The travel time of the water from the surface provided in the database (determined by hydrogen isotope ratio) was <30 a, if available.
- no water infiltration from rivers
- permeable topsoil
- restricted to the areas where crops which are treated with bentazone, i.e., cereals and maize, were grown on ≥10 % of land
- relevant land use at the time of well installation

A clear assignment of a catchment area for single wells could not be performed, but as the monitored groundwater is assumed to represent integration over several years of groundwater recharge, detection of bentazone leaching is possible if crops to which bentazone is applied are grown in the surrounding of several kilometers around the monitoring well.

As far as information was available beforehand, all wells had been properly drilled by professional drilling companies and the well pipe properly sealed, e.g. with clay, at the top part as well as at every impermeable soil layer. The wells were single filter wells with a filter length of 2 m. In most cases two or three wells of different depths were installed into one borehole. The inner diameter of the sampling pipe was mostly 2 inch, in some cases 1 inch.

4. Sampling and analysis

Before and during sampling, the groundwater level was measured with a light gauge. The water samples were taken with a suitable pump (e.g. submersible or suction pump), depending on the diameter of the filter tube. The pump rate was adjusted to the capacity of the well and a total pre-pumping duration of at least 30 min (non-GLP) was used. During this pre-pumping, water was pumped with a delivery rate of approximately 0.1 L/s, and measurements of temperature, pH, electrical conductivity and O_2 concentration (all non-GLP) were measured in a flow-through cell.
Sampling was at approximately 0.1 L/s, unless the conditions (e.g. well capacity) required an adaption of this rate, and was initiated when the measured figures varied no more than approximately 10%, or when the pre-pumping had delivered more than estimated 50 volumes of the well pipe.

The crops grown in the direct vicinity of the well in the current growing season as well as possible groundwater contamination by irrigation wells in the vicinity or by overspray/infiltration were recorded at the time of sampling.

From each well at least 2 L water sample was filled into a brown glass bottle with a screw top, avoiding intake of air bubbles into the bottles. The samples were, immediately after sampling, stored in cooling boxes at an average temperature of below 6 °C in the dark during transport. The samples were directly delivered to the sample management laboratory. From receipt to final processing the samples were stored at 5 °C.

All water samples were analysed for bentazone residues with GC/MS according to BASF method no. 423 (*Keller, W. (1998): Validation of analytical method no. 423, determination of Bentazone residues in water, BASF DocID 1998/10079*). This method is based on the enrichment of bentazone from acidified water by adsorption on a C18-SPE column. After desorption of the bentazone residues from the SPE column with acetone and the methylation with diazomethane, the formed N-methyl-bentazone is cleaned up by silica gel column chromatography. The final quantitative determination of N-methyl-bentazone is performed by GC/MS. The method has been validated for the analysis of tap water and surface water samples, with a limit of quantitation of 0.05 µg/kg.
Positive results close to the limit of quantitation were confirmed by an additional analysis.

5. Method validation

Within each analytical series procedural recovery experiments were performed to prove the reliability of the analytical method. The recovery samples were prepared with tap water fortified with bentazone at different concentration levels. Tap water was used for the control samples.

To check for potential matrix effects, one ground water sample slightly yellow in colour with no detectable bentazone residues was used to prepare fortified samples.

II. RESULTS AND DISCUSSION

A. Method validation

The mean recovery of all fortified samples was 91.9 ± 7.6 % (± 1 SD, n = 42). The samples prepared with slightly yellow ground water showed recovery results in a similar range as the tap water fortified samples. Therefore it can be concluded that the matrix has no significant influence on the performance of the analytical method. These results are also in good agreement with the validation data obtained with surface water.

The good agreement of replicate analytical results and the high recoveries prove that the analytical method applied is able to accurately determine bentazone residues in groundwater.

The unfortified control samples were free of bentazone residues, indicating that no interferences occurred during the analytical procedure.

B. Findings

Many wells had been installed next to drainage ditches. As the ditches shortcut the topsoil as the shield of groundwater they pose an increased risk to groundwater, since surface water may enter groundwater during annual water level fluctuations. Therefore the placement of those wells represented a worst case for the results of the monitoring. Moreover, very often subsurface wellheads or streetpots had been used. In many cases they were improperly placed and not sealed, so surface runoff could easily enter the filter tubes.

A summary of the observed groundwater concentrations as well as the derivation of a 90th percentile concentration of bentazone in Dutch groundwater is given Table 7.12/18. Concentrations lower than the LOQ were set to a value of half the LOQ concentration. Concentrations of repeated measurements are given as arithmetic mean. Concentrations used for derivation of the 90th percentile are listed in the last column (excludes measurements for 10 deselected well filters; reasons for de-selection are given in the table).

The 90th percentile of bentazone concentrations in groundwater at 10 m depth was 0.025 µg/L (n=92).

Table 7.12/18 Derivation of a 90th percentile concentration in Dutch groundwater

Study site	Sampling no.	Bentazone residue [µg/kg]	Assumed concentration* for calculation of percentile [µg/L]	Well filter deselected from consideration		Concentration values relevant for derivation of 90th percentile [µg/L]
O	1	< 0.050	0.025			0.025
O	2	< 0.050	0.025			0.025
O	3	< 0.050	0.025			0.025
O	4	< 0.050	0.025			0.025
O	5	< 0.050	0.025			0.025
O	6	< 0.050	0.025			0.025
O	7	< 0.050	0.025			0.025
O	8	< 0.050	0.025			0.025
O	9	< 0.050	0.025			0.025
O	10	< 0.050	0.025			0.025
O	11	< 0.050	0.025			0.025
O	12	< 0.050	0.025			0.025
O	13	0.105	0.105			0.105
O	14	< 0.050	0.025			0.025
O	15	0.110	0.110			0.110
O	16	< 0.050	0.025			0.025
O	17	< 0.050	0.025			0.025
O	19	< 0.050	0.025			0.025
O	20	< 0.050	0.025			0.025
O	21	< 0.050	0.025			0.025
O	22	< 0.050	0.025			0.025
O	23	< 0.050	0.025			0.025
O	24	< 0.050	0.025			0.025
O	25	< 0.050	0.025			0.025
O	26	0.583	0.583			0.583
O	27	< 0.050	0.025			0.025
O	29	< 0.050	0.025	+	No Bentazone crops in the vicinity of 1 km around well	
O	30	< 0.050	0.025	+	Streetpot. Runoff water can enter the wellhead. As the top end of the filter tube of the deeper filter is ca. 10 cm lower than that of filter 1 the runoff water will first enter the deeper filter and contaminate the groundwater. The deeper well (34 O) shows contamination, the shallower does not (30 O).	
O	31	0.098	0.098			0.098
O	32	< 0.050	0.025			0.025
O	33	< 0.050	0.025	+	No Bentazone crops in the vicinity of 1 km around well	
O	34	0.099	0.099	+	Streetpot. Runoff water can enter the wellhead. As the top end of the filter tube of the deeper filter is ca. 10 cm lower than that of filter 1 the runoff water will first enter the deeper filter and contaminate the groundwater. The deeper well (34 O) shows contamination, the shallower does not (30 O).	

*Values of <LOD were set to 0.025 (=half of LOQ)

Table 7.12/18 continued

Study site	Sampling no.	Bentazone residue [µg/kg]	Assumed concentration* for calculation of percentile [µg/L]	Well filter deselected from consideration		Concentration values relevant for derivation of 90th percentile [µg/L]
O	35	< 0.050	0.025			0.025
O	36	< 0.050	0.025			0.025
O	37	< 0.050	0.025			0.025
O	38	< 0.050	0.025			0.025
O	39	< 0.050	0.025			0.025
O	40	< 0.050	0.025			0.025
O	42	< 0.050	0.025			0.025
O	43	< 0.050	0.025			0.025
O	44	< 0.050	0.025			0.025
O	45	0.688	0.688			0.688
O	46	< 0.050	0.025			0.025
O	47	0.063	0.063	+	Filter was accidentally selected, even if it is 30m deep.	
O	48	< 0.050	0.025			0.025
O	49	< 0.050	0.025			0.025
O	50	< 0.050	0.025			0.025
O	51	< 0.050	0.025			0.025
O	52	0.271	0.271	+	Wellhead was damaged (ca. 15° skewed). So the clay seal will be damaged also.	
O	53	< 0.050	0.025			0.025
O	54	< 0.050	0.025			0.025
O	56	< 0.050	0.025			0.025
O	57	< 0.050	0.025			0.025
O	58	< 0.050	0.025			0.025
O	59	< 0.050	0.025			0.025
O	60	< 0.050	0.025			0.025
O	61	< 0.050	0.025			0.025
O	62	< 0.050	0.025			0.025
O	63	< 0.050	0.025	+	Wellhead was damaged (ca. 15° skewed). So the clay seal will be damaged also.	
O	64	< 0.050	0.025			0.025
O	66	< 0.050	0.025			0.025
O	67	< 0.050	0.025			0.025
O	68	0.322	0.322	+	Dark flakes, probably of organic origin, occurred in samples. As drilling profile shows pure sand at the sampling depth, filter tube must be damaged in the top soil zone.	
O	69	< 0.050	0.025			0.025
O	70	< 0.050	0.025			0.025
O	71	< 0.050	0.025			0.025
O	72	< 0.050	0.025			0.025
O	73	< 0.050	0.025			0.025
O	74	< 0.050	0.025			0.025

*Values of <LOD were set to 0.025 (=half of LOQ)

Table 7.12/18 continued

Study site	Sampling no.	Bentazone residue [µg/kg]	Assumed concentration* for calculation of percentile [µg/L]	Well filter deselected from consideration		Concentration values relevant for derivation of 90th percentile [µg/L]
S	1	< 0.050	0.025			0.025
S	3	0.228	0.228	+	Bank filtrate from Gent-Terneuzen canal not far from Westerschelde, influence of seawater	
S	4	< 0.050	0.025			0.025
S	5	< 0.050	0.025			0.025
S	6	< 0.050	0.025			0.025
S	7	< 0.050	0.025			0.025
S	8	< 0.050	0.025			0.025
S	9	< 0.050	0.025			0.025
S	10	< 0.050	0.025			0.025
S	11	< 0.050	0.025			0.025
S	12	< 0.050	0.025			0.025
S	13	< 0.050	0.025			0.025
S	14	< 0.050	0.025			0.025
S	15	< 0.050	0.025			0.025
S	16	< 0.050	0.025			0.025
S	17	< 0.050	0.025			0.025
S	18	< 0.050	0.025			0.025
S	19	< 0.050	0.025	+	No Bentazone crops around well	
S	20	< 0.050	0.025			0.025
S	21	< 0.050	0.025			0.025
S	22	< 0.050	0.025			0.025
S	23	< 0.050	0.025			0.025
S	24	< 0.050	0.025			0.025
S	25	< 0.050	0.025			0.025
S	26	0.051	0.051			0.051
S	27	< 0.050	0.025			0.025
S	28	0.095	0.095			0.095
S	29	< 0.050	0.025			0.025
S	30	< 0.050	0.025			0.025
S	31	< 0.050	0.025			0.025
S	32	< 0.050	0.025			0.025
S	33	< 0.050	0.025			0.025
S	34	< 0.050	0.025			0.025
Number of wells			102	10	**90th percentile concentration**	**0.025**

*Values of <LOD were set to 0.025 (=half of LOQ)

III. CONCLUSION

Carefully selected representative groundwater wells were sampled in relevant agricultural regions of the Netherlands to assess the risk of groundwater contamination through bentazone.

The 90th percentile (n=92) of bentazone concentrations in groundwater was 0.025 µg/L; therefore the use of bentazone poses no risk for the contamination of groundwater in 10 m depth.

Report:	II A 7.12/6 Loos R. et al. 2010(a) Pan-European survey on the occurrence of selected polar organic persistent pollutants in ground water BASF DocID 2010/1227002
Guidelines:	None
Testing Laboratory and dates:	22-May-2010
GLP:	No, not subject to GLP regulations (scientific publication)

Executive Summary

This groundwater monitoring study was initiated by the Joint Research Centre's Institute for Environment and Sustainability (Ispra, Italy) and investigates the occurrence of polar organic persistent pollutants in European groundwater. It is explicitly stated by the authors that the study should be seen as comprehensive picture ("snapshot") of typical ground water scenarios in Europe and should not be considered as a statement of the ground water quality in the Member States or as a characterisation of a single sampling station. Several European regions were under-represented (e.g. Germany, France, Spain). But on a continent scale, the authors considered the sample pool as "good" for the overall European groundwater situation.

In total, 164 groundwater samples from 23 European countries were collected and analysed for 59 selected organic compounds, comprising pharmaceuticals, antibiotics, pesticides (and their transformation products), perfluorinated acids (PFAs), benzotriazoles, hormones, alkylphenolics (endocrine disrupters), Caffeine, Diethyltoluamide (DEET), and Triclosan.

Bentazone was analysed with a LOD of 0.4 ng/L. It was detected in 32% of the samples but exceeded the groundwater standard of 0.1 µg/L in only 4 out of the 164 samples. The maximum value detected in a single well was 10.55 µg/L. No explanation for such an unusual high value was given in the publication. The authors calculated the average concentrations including this high value which resulted then in a high average of 0.116 µg/L. The median was calculated with 0 µg/L and the 90th percentile with 0.015 µg/L.

I. MATERIAL AND METHODS

Sampling and transport

The investigated ground water monitoring stations were proposed upon invitation by the individual participating EU Member State laboratories and finally selected by the JRC. The authors mentioned that the sampling sites were not selected according to being "representative“ or “contaminated“; but most monitoring stations were “official“ monitoring stations also used for drinking water abstraction. The sampling took place in a time window of 8 weeks in autumn 2008. JRC provided the pre-cleaned and conditioned sample containers to the participating laboratories and sampling teams.The participants were asked to fill the bottles leaving a small air head-space. The containers were dispatched by fast courier under cooled conditions (4 °C in cooling boxes) within 48h to the JRC Ispra Site (Italy) for further processing. In total, 164 European groundwater sampling stations were screened.

Sample workup

The water samples were extracted at the JRC by solid-phase extraction (SPE). Before extraction, the samples (1 L) were spiked with the internal standard (50 µL), which contained the labelled substances PFOA $^{13}C_4$, PFOS $^{13}C_4$, PFNA $^{13}C_5$, Carbamazepine db, Simazine $^{13}C_3$, Atrazine $^{13}C_3$, Ibuprofen $^{13}C_3$, Nonylphenol $^{13}C_6$, Octylphenol $^{13}C_6$, Estrone d2, 2,4-D d3, MCPA d3, and Triclosan $^{13}C_{12}$. The spiking level in the water samples was 5 ng/L for PFOA $^{13}C_4$, PFOS $^{13}C_4$, PFNA $^{13}C_5$, Octyl- and Nonylphenol $^{13}C_6$, and 50 ng/L for the other labeled compounds. The glass bottles were closed, and then the samples were mixed by shaking. The cleanup and concentration via SPE was performed automatically using an AutoTrace® SPE workstation.

Liquid chromatography tandem mass spectrometry (LC-MS/MS)

Analyses were performed by reversed-phase liquid chromatography (RP-LC) followed by electrospray ionization (ESI) mass spectrometry (MS) detection using atmospheric-pressure ionization (API) with a triple-quadrupole MS—MS system. Quantitative LC-MS/MS analysis was performed in three separate LC-MS/MS runs (methods 1-3) in the multiple reaction monitoring (MRM) mode. Method 1 comprised the compounds in the negative ionization mode, method 2 those in the positive ionization mode and method 3 alkylphenolic compounds and estrogens, which were analysed with a different HPLC mobile phase.

Analytical quality control

The absolute recoveries for the chemicals including the internal standards were determined with spike experiments in the concentration range of 10 and 100 ng/L using Milli-Q water (replication n=6); they were in the range of 50-90%. The limits of detection (LODs) for the SPE-LC-MS/MS procedure were calculated from the mean concentration of the blank of real water samples plus three times the standard deviation. The measurement uncertainty is estimated to be around 25—50%. In addition, in 2009 the authors participated in the 3rd inter-laboratory study on perfluorinated compounds in water, fish, and sludge (organized by Stefan van Leeuwen, Institute for Environmental Studies (IVM), VU University Amsterdam, NL). Moreover, the samples from Austria were cross-checked by Umweltbundesamt Vienna for some compounds such as Bentazone, Atrazine, Terbutylazine, and Sulfamethoxazole.

II. RESULTS AND CONCLUSION

In total, 59 different organic chemical compounds were analysed. Since the limit of detection was in the low ng/L range for most chemicals, there was no sample free of organic chemicals. There were a few samples where only 3 compounds were found, but there were also samples with higher compound numbers. The maximum number found in a single well was 29.

Bentazone was analysed with a LOD of 0.4 ng/L. It was detected in 32% of the samples but exceeded the groundwater standard of 0.1 µg/L in only 4 out of the 164 samples. The maximum value detected in a single well was 10.55 µg/L. No explanation for such an unusual high value was given in the publication. The authors calculated the average concentrations including this high value which resulted then in a high average of 0.116 µg/L. The median was calculated with 0.0 µg/L and the 90th percentile with 0.015 µg/L.

Overall conclusion concerning monitoring data

Surface water
Monitoring data from many different rivers in different countries of Europe carried out by EU authorities as well as BASF showed values that are far below the level of toxicological or ecotoxicological concern.

Groundwater

Different monitoring studies were carried out either as targeted monitoring, where the use of bentazone near the well was documented, or as non-targeted monitoring, where it was not known if bentazone was used in the catchment area. The common result of all studies was that the 90th percentile of the values was clearly below the drinking water threshold of 0.1 µg/L.

Furthermore, the BASF trend analysis concerning groundwater findings in Germany showed that for the majority of individual wells for which a trend analysis by linear regression methods was possible, a downward trend was identified.

7.13 Other/special studies

Report: II A 7.13/1
Ciucani G. et al. 2002(a)
Measurement of xylem translocation of weak electrolytes with the pressure chamber technique
BASF DocID 2002/1025760
Guidelines: None
Testing Laboratory and dates: 22-Jan-2002
GLP: No, not subject to GLP regulations (scientific publication)

Executive Summary

In this scientific publication, xylem transportation and root uptake of weak electrolytes were determined with the pressure chamber technique using de-topped soybean plants. One of the compounds investigated was bentazone. The transpiration stream concentration factors (TSCF) and root concentration factors (RCF) were determined from the concentrations in the external solution and in the xylem sap at different pH values in the external solution (pH 4.5, 6.5 and 8.5). For the weak acid bentazone the TSCF values were in the range of 0.69-0.92 and the RCF values in the range of 25 to 84.

I. MATERIAL AND METHODS

1. Test Material

Bentazone (purity 99%) was supplied by Riedel-de Haën.

2. Experimental conditions

Soybean plants (*Glycine max* L, 'Cresir') and pressure-chamber technique (PCT) were used following an established method. Soybean seeds were germinated in the dark on paper towelling saturated with water at a constant temperature of 26°C. Five days later, seedlings were transferred to 10-liter plastic containers for hydroponic cultivation in Hoagland solution, and were kept in a glasshouse (26°C day, 18°C night).

Plants were 20-27 days old, between R1 and R2 early flowering stage when used for experiments. They were excised just below the cotyledonary node and put into the beaker inside the pressure chamber. Root uptake and xylem transport were measured at pH values in external solution of 4.5, 6.5 and 8.5. The whole root was bathed in a solution buffered with 50 mM MES-TRIZMA (2-N-morpholinoethanesulfonic acid and Tris-hydroxymethylaminomethane) and containing the test compound at a concentration of 0.5 mg/L (measured by HPLC using calibration with external standard). Hydrostatic pressure (0.3-0.4 MPa), generated by compressed air containing 7% oxygen, was applied to the root and held constant.

The solution containing the root was constantly aerated by an air pump. After a few minutes a constant xylem sap flow (0.1 mL/min) was obtained. The experiments were run at least in four replicates for most compounds and for 6 h. One experiment was run for 15 h with every compound.

3. Description of analytical procedures

Xylem sap was injected directly into a Hewlett- Packard 1100 HPLC instrument equipped with a diode-array detector and fitted with a Phenomenex C18 (2) Luna (250 mm x 4.60 mm, 5 mm) column. In these conditions the minimum concentration detectable was 0.05 mg/L. The injector loop was 100 µL; flow rate was 1 mL/min; wavelength and eluent mixtures were chosen according to the literature and the best chromatographic performance of each compound. The concentration of active ingredients was determined under gradient conditions. The mobile phase was CH_3CN/buffer NaH_2PO_4/H_3PO_4 (0.02 M) pH 3 from 50/50 to 67/33 in 19 min.

4. Calculation of results

From the measured concentrations in xylem sap, the transpiration stream concentration factor TSCF was calculated:

$$\mathrm{TSCF} = \frac{\text{concentration in xylem at the steady-state efflux } (\mu g\ ml^{-1})}{\text{concentration in the external solution } (\mu g\ ml^{-1})}$$

Ideally, TSCF expresses the ratio between the chemical concentration in xylem sap (C_{XY}) and in external solution (C_S).

The equation

$C_{xy} = A \exp(-k_2 t) \{1-\exp(-k_1 t)\}$

was least-square fitted to the data, where C is the estimate of the measured concentrations in xylem sap. If the system is linear, the fit is good and the coefficients can be physically interpreted:

$A = TSCF/C_s$

$k_1 = \ln 2/t_{50}$

where t_{50} is the time when the steady-state TSCF reaches 50%. The coefficient k_2 can account for losses from the external solution, eg by root uptake, translocation and metabolism. It was only fitted when necessary (zero otherwise) to keep the number of fit parameters small. The concentration in external solution C changed during the experiment; the mean of initial and final measured concentration was used.
Uptake into roots and shoots was not measured directly, but calculated from the loss of chemical from external solution:

Amount in roots = initial amount in external solution
- final amount in external solution
- amount lost by translocation in the xylem

The root concentration factor RCF was then calculated from die virtual concentration in roots divided by the concentration in solution. RCF values such as those given below can only indicate a tendency, and do not necessarily represent real concentrations in roots, since every loss is attributed to uptake into plants. Additional loss processes are uptake into stem, metabolism inside plants, volatilisation and loss by handling. Loss due to sorption to glass walls and abiotic degradation processes could be excluded by measuring controls without plants.

II. RESULTS AND DISCUSSION

The TSCF values calculated for bentazone using the pressure chamber technique are given in Table 7.13/1. The root concentration factors (RCF) are given in Table 7.13/2.The authors remark that TSCF values differ only slightly with pH, whereas RCF values show a larger variability.

Table 7.13/1 Fitted coefficients and derived TSCF and t50 values for bentazone at different pH values

pH	A	k_1 (min^{-1})	k_2 (min^{-1})	LSE	TSCF	t_{50} (min)
4.5	0.63	0.06	0.0009	0.012	0.92	11.6
6.5	0.38	0.18	0	0.022	0.78	3.9
8.5	0.39	0.18	0	0.009	0.69	3.9

(LSE = least square error of the fit)

Table 7.13/2 Root conc. factor values for bentazone at different pH values

pH	RCF
4.5	25 (±4)
6.5	65 (±1)
8.5	84 (±31)

The authors also admit that differences between the determination of the TSCF in intact plants and by use of the pressure chamber technique are observable. By use of the pressure chamber the pH difference between xylem sap in the de-topped plants and external solution was less than 0.5 pH units, whereas in intact plants the pH in the xylem apoplast is kept in a range of 5 and 6.5. The xylem pH strongly influences translocation of weak electrolytes. The accumulation of weak electrolytes in the xylem apoplast is driven by the ion trap effect. That means that weak acids accumulate when the pH in the external solution is below that in the xylem, but are excluded in the opposite direction. The authors admit that the lack of pH differences between external solution and xylem might explain that the measured TSCF values are rather close to 1 but not higher at higher pH as would probably be the case in whole plant systems.

III. CONCLUSION

The xylem transport and root uptake of bentazone was characterized in solutions of different pH in de-topped soybean plants using the pressure chamber technique. The transpiration stream concentration factors (TSCF) and root concentration factors (RCF) were calculated and found to be in the range of 0.69 to 0.92 for the TSCF and 25 to 84 for the RCF.

BASF
The Chemical Company

DOSSIER FOR THE EVALUATION OF THE ACTIVE SUBSTANCE

Bentazone (BAS 351 H)

Data requirement according to Regulation (EU) No. 544/2011 and (EU) No. 545/2011 (formerly Annex II and III of Directive 91/414/EEC) using OECD Guidance for Industry Data Submissions on Plant Protection Products and their Active Substance (Dossier Guidance)

Document M-II

Summary and evaluation (Tier II)

Section 6

Ecotoxicological studies on the active substance

BASF DocID 2014/1046511

compiled by

[redacted]

Date: 03 February 2014

8 Ecotoxicological Studies on the Active Substance

List of Contents

According to Article 1(c) of Regulation (EU) No. 1141/2010 the supplementary dossier includes data and risk assessments which were not part of the original dossier and which are necessary to reflect changes. Reasoning for each test or study as required by Art 1(d)/(e) of the Regulation is given in the reference list of the dossier.

According to Article 1(f) of Regulation (EU) No. 1141/2010 the supplementary dossier shall include a description of the steps taken to avoid animal testing and duplication of tests and studies on vertebrate animals for each test or study involving vertebrate animals.

All vertebrate studies have been conducted in order to fulfil the data requirements for the re-approval of the active substance and the authorisation of plant protection products. Furthermore, the test strategy was based on corresponding guidance documents takes into account methods compliant with the 3R concept for refinement, reduction and replacement of animal testing where applicable and acceptable.

In order to avoid duplication of tests and studies BASF has submitted a list of studies to be generated within the application. This information has been accessible to other co-notifiers.
Vice versa BASF had access to the respective information as provided by other co-notifiers.
In addition BASF has performed a literature search.
However, generally it has been assumed that BASF as the main data owner for bentazone will be contacted by co-notifiers before initiating vertebrate studies.

For Bentazone a literature search has been performed. The search report including a description of the selection and assessment process is provided in BASF DocID 2012/1007281 (DocLIIA).
The results of the selection process and assessments for Ecotoxicological studies are outlined in BASF DocID 2012/1007284.

8.1 Avian toxicity

8.1.1 Acute oral toxicity to quail species, mallard duck or other bird

An avian acute toxicity study on bobwhite quail (*Colinus virginianus*) was performed with bentazone ([redacted], 1986, BASF DocID 1986/9002) as single-dose test. This study was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

In the avian single-dose acute toxicity study with bobwhite quail the LD_{50} of bentazone was about 1140 mg a.s./kg b.w. The NOEL was determined to be 500 mg a.s./b.w.

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996) and in the EC Review Report (7585/V1197-final, November 2000) for bentazone.

8.1.2 Avian dietary toxicity (5-day) test in quail species or mallard duck

A 5-day avian dietary (short-term dietary) toxicity study on chicks of bobwhite quail (*Colinus virginianus*) was performed with bentazone [redacted], 1986, BASF DocID 1986/9003) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

In a short-term avian dietary study with bobwhite quail the LC_{50} of bentazone was > 5000 mg a.s./kg diet.

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996) and in the EC Review Report (7585/V1197-final, November 2000) for bentazone.

Note that for risk assessments conducted according to current bird and mammal guidance EFSA/2009/1438 the short-term exposure scenario is not required and therefore TER short-term values are not calculated in Document M-III, Chapter 10 01.

8.1.3 Avian dietary toxicity (5-day) test in a second unrelated species

A 5-day avian dietary (short-term dietary) toxicity study on chicks of mallard duck (*Anas platyrhynchos*) was performed with bentazone [redacted] 1986, BASF DocID 1986/9000) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

In a short-term avian dietary study with mallard duck the LC_{50} of bentazone was > 5000 mg a.s./kg diet.

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996) and in the EC Review Report (7585/V1197-final, November 2000) for bentazone.

Note that for risk assessments conducted according to current bird and mammal guidance EFSA/2009/1438 the short-term exposure scenario is not required and therefore TER short-term values are not calculated in Document M-III, Chapter 10 01.

8.1.4 Subchronic and reproductive toxicity to birds

Report:	II A 8.1.4/1 [redacted] 1997(c) Bentazon: A definitive reproduction study with the mallard (Anas platyrhynchos) BASF DocID 1997/5306
Guidelines:	EPA 71-4; OECD 206; EPA-Guideline - Pesticide Assessment Guidelines. Subdiv. E Hazard Evaluation Wildlife and Aquatic Organisms **Deviations:** None
GLP:	Yes (laboratory certified by United States Environmental Protection Agency)

In a 22-week avian reproduction test with mallard duck (*Anas platyrhynchus,* BASF DocID 1997/5306), no substance-related adverse effects on mortality, health, palatability, food consumption and body weight of the parental generation could be detected up to 800 mg a.s./kg diet, the highest dose in the test. The NOEC = 800 mg a.s./kg diet was confirmed in Review report for the active substance bentazone, 7585/VI/97-final, 30.November 2000.

To calculate the mean daily dose, the concentration in diet (mg a.s./kg diet) was multiplied by the daily food consumption (g per bird per day) and divided by the body weight. The results are summarized in Table 8.1/1

Table 8.1/1 Mean daily dose of mallard ducks (*Anas platyrhynchus*) exposed to bentazone

Parameter	Treatment group [mg a.s./kg diet]			
	100	**400**	**600**	**800**
Nominal concentration in diet [mg a.s./kg diet]	100	400	600	800
Mean food consumption [g/bird/day] [1)]	162.45	152.73	151.82	175.73
Mean adult body weight [2)] [g]	1132.50	1092.42	1094.92	1093.00
Mean daily dose [mg a.s./kg body weight] [3)]	14	56	83	129

1) Calculated mean value for week 1 to week 22. For detailed values see Table 2, p. 31 in BASF DocID 1997/5306
2) Calculated mean of values for males and females as reported for week 0, week 2, week 4, week 6, week 8 and adult termination. For detailed values see Table 1, p. 28, in BASF DocID 1997/5306
3) Mean daily dose (mg a.s./kg b.w./d) = concentration in diet (mg a.s./kg diet) multiplied by the daily food consumption (g per bird per day) and divided by the body weight.

CONCLUSION

In a 22-week avian reproduction test with mallard ducks (Anas platyrhynchus), no substance-related adverse effects on mortality, health, palatability, food consumption and body weight of the parental generation could be detected up to 800 mg a.s./kg diet, the highest dose in the test.

The NOEC for reproduction of bentazone was determined to be 800 mg a.s./kg diet corresponding to a NOEL of 129 mg a.s./kg body weight.

Report: II A 8.1.4/2
[redacted] 1997(c)
Bentazon: A definitive reproduction study with the Northern bobwhite (Colinus virginianus)
BASF DocID 1997/5007

Guidelines: EPA 71-4; OECD 206; EPA-Guideline - Pesticide Assessment Guidelines. Subdiv. E Hazard Evaluation Wildlife and Aquatic Organisms
Deviations: None

GLP: Yes
(laboratory certified by United States Environmental Protection Agency)

In a 22-week avian reproduction test with bobwhite quails (*Colinus virginianus,* BASF DocID 1997/5007), no substance-related adverse effects on mortality, health, palatability, food consumption and body weight of the parental generation could be detected up to 800 mg a.s./kg diet, the highest dose in the test. The NOEC = 800 mg a.s./kg diet was confirmed in Review report for the active substance bentazone, 7585/VI/97-final, 30.November 2000.

To calculate the mean daily dose, the concentration in diet (mg a.s./kg diet) was multiplied by the daily food consumption (g per bird per day) and divided by the body weight. The results are summarized in Table 8.1/2

Table 8.1/2 Mean daily dose of bobwhite quails *(Colinus virginianus*) exposed to bentazone

Parameter	Treatment group [mg a.s./kg diet]			
	100	**400**	**600**	**800**
Nominal concentration in diet [mg a.s./kg diet]	100	400	600	800
Mean food consumption [g/bird/day] [1)]	18.45	18.59	19.86	20.00
Mean adult body weight [2)] [g]	211.17	211.58	211.75	212.08
Mean daily dose [mg a.s./kg body weight/d] [3)]	8.74	35.15	56.28	75.44

1) Calculated mean value for week 1 to week 22. For detailed values see Table 2, p. 30 in BASF DocID 1997/5007
2) Calculated mean of values for males and females as reported for week 0, week 2, week 4, week 6, week 8 and adult termination. For detailed values see Table 1, p. 27, in BASF DocID 1997/5007
3) Daily dose (mg a.s./kg b.w./d) = concentration in diet (mg a.s./kg diet) multiplied by the daily food consumption (g per bird per day) and divided by the body weight.

CONCLUSION

In a 22-week avian reproduction test with bobwhite quails (*Colinus virginianus*), no substance-related adverse effects on mortality, health, palatability, food consumption and body weight of the parental generation could be detected up to 800 mg a.s./kg diet, the highest dose in the test.

The NOEC for reproduction of bentazone was determined to be 800 mg a.s./kg diet corresponding to a NOEL of 75.44 mg a.s./kg b.w./d.

8.2 Fish toxicity

8.2.1 Acute toxicity of the active substance to fish

8.2.1.1 Rainbow trout (*Oncorhynchus mykiss*)

A fish acute toxicity study on *Oncorhynchus mykiss* was performed with bentazone ([redacted] 1987, BASF DocID 1987/0428) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

In the static acute toxicity study with rainbow trout the LC_{50} (96 h) of bentazone was > 100 mg a.s./L based on nominal concentrations. The NOEC (96 h) was determined to be 50 mg a.s./L (nominal).

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996) and in the EC Review Report (7585/VI/97-final, November 2000) for bentazone.

8.2.1.2 Warm water fish species

An acute toxicity study on *Pimephales promelas* has been performed with the Na-salt of bentazone. It is reported here as it contains relevant information and was only recently finalized and not submitted yet in an Annex II dossier.

Reference number:	II A 8.2.1.2/1
Report:	[redacted] 2011(a) Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) - Acute toxicity study in the fathead minnow (Pimephales promelas) [redacted] unpublished BASF DocID 2011/1173854
Guidelines:	EC 440/2008 C.1 Acute Toxicity for Fish; OECD 203 (1992); EPA 540/9-82-024; EPA 850.1075; ISO 7346 - 1996 (E); OECD Series on Testing and Assessment No. 23 (2000) - Aquatic Toxicity Testing of Difficult Substances and Mixtures; EPA 712-C-96-118
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a static acute toxicity laboratory study, fathead minnow (*Pimephales promelas*, approx. 2 months old) were exposed to a control and to 120 mg bentazone-Na/L (nominal) in groups of 10 animals in glass aquaria containing 10 L water with 3 replicates for the test item treatment and 2 replicates for the control. Fish were observed for survival and symptoms of toxicity within 1 hour after start of exposure and 6, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on the nominal concentration. After 96 hours of exposure no mortality and no toxic effects were observed in the control and at the tested concentration of 120 mg bentazone-Na/L.

In a static acute toxicity study with fathead minnow the LC_{50} (96 h) for bentazone-Na was determined to be > 120 mg/L, based on the nominal concentration. The NOEC (96 h) was ≥ 120 mg/L (nominal).

I. MATERIAL AND METHODS

Test item:	Bentazone-Na (BAS 351 H-Na, Reg. no. 88 691; B), batch no. COD-001417, purity: 91.9% (tolerance ± 1.0%).
Test species:	Fathead minnow (*Pimephales promelas*), approx. 2 months old; mean body length 1.9 cm (1.5 - 2.2 cm), mean body weight 0.12 g (0.06 - 0.17 g); fish were bred and hatched in-house.
Test design:	Static system (96 hours); 10 fish per aquarium (loading 0.12 g fish/L), 3 replicates for the test item treatment and 2 replicates for the control; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and 6, 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Control, 120 mg a.s/L (nominal concentrations based on test substance without correction for purity).
Test conditions:	10 L glass aquaria with stainless steel frames (29 x 21 x 22 cm), test volume 10 L, non-chlorinated, filtered tap water, mixed with deionized water; temperature: 23 °C ± 1 °C; pH 8.2 - 8.3; oxygen content: 6.7 mg/L - 8.5 mg/L; total hardness: about 100 mg $CaCO_3$/L; conductivity: approx. 250 µS/cm^2; photoperiod 16 h light : 8 h dark; light intensity: 96 lux - 451 lux; no aeration, no feeding.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test substance concentration was conducted in each replicate at test initiation, after 48 hours of exposure and at the end of the test. All measured concentrations deviated less than 20% from the nominal concentration. Therefore exposure conditions were consistent and results can be expressed relative to the nominal concentration.

Biological results: After 96 hours of exposure no mortality and no toxic effects were observed in the control and at the tested concentration of 120 mg bentazone-Na/L. The results are summarized in Table 8.2/1.

Table 8.2/1 Acute toxicity (96 h) of bentazone-Na to fathead minnow (*Pimephales promelas*)

Concentration [mg/L] nominal	**Control**	**120**
Mortality [%]	0	0
Symptoms	none	none
	Endpoints [mg a.s./L] (nominal)	
LC_{50} (96 h)	> 120	
NOEC (96 h)	≥ 120	

III. CONCLUSION

In a static acute toxicity study with fathead minnow the LC_{50} (96 h) for bentazone-Na was determined to be > 120 mg/L, based on nominal concentrations. The NOEC (96 h) was ≥ 120 mg/L (nominal).

Additionally. a fish acute toxicity study on *Lepomis macrochirus* was performed with bentazone [redacted], 1986, BASF DocID 1986/9005) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

In the static acute toxicity study with bluegill sunfish the LC_{50} (96 h) of bentazone was > 100 mg a.s./L based on nominal concentrations. The NOEC (96 h) was determined to be 100 mg a.s./L (nominal).

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996).

Additionally, a fish acute toxicity study on *Cyprinus carpio* was performed with the Na-salt of bentazone ([redacted] 1983, BASF DocID 1983/10048) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

In the static acute toxicity study with common carp the LC_{50} (96 h) of bentazone-Na was > 1000 mg/L based on nominal concentrations. The NOEC (96 h) was determined to be 560 mg/L (nominal).

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996).

Additionally, a fish acute toxicity study on *Brachydanio rerio* was performed with bentazone (tested with the lysimeter leachate of the formulation BAS 351 32 H; [redacted] 1994, BASF DocID 1994/11076) and was assessed during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

All fish in the labeled undiluted lysimeter perculates or in the undiluted lysimeter control or in the municipal water control survived the test period of 96 hours (= 4 days) without any visible signs of toxicity and/or unusual behaviour.

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996).

8.2.1.3 Acute toxicity of metabolites to the more sensitive of fish species

An acute toxicity study on *Oncorhynchus mykiss* has been performed with the bentazone metabolite N-methyl bentazone (= M351H009). It is reported here as it contains relevant information and was only recently finalized and not submitted yet in an Annex II dossier.

Reference number:	II A 8.2.1.3/1
Report:	[redacted] 2004(b) Reg.No. 79520 (metabolite of BAS 351 H) - Acute toxicity study on the rainbow trout (Oncorhynchus mykiss) in a static system over 96 hours [redacted] unpublished BASF DocID 2004/1025175
Guidelines:	EPA 72-1; EPA 850.1075; EPA-SEP 540/9-85-006; OECD 203; EEC 92/69 A V C 1
GLP:	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a static acute toxicity laboratory study, juvenile rainbow trout (approx. 4 months old) were exposed to 5, 10, 22, 50 and 100 mg N-methyl-bentazone/L (= M351H009; nominal) in groups of 10 animals in glass aquaria containing 50 L water with 2 replicates per concentration. Fish were observed for survival and symptoms of toxicity within 1 hour after start of exposure and 4, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on the mean measured concentrations. After 96 hours of exposure no mortality and toxic effects were observed in the control and at the concentrations of 5 mg N-methyl-bentazone/L, whereas 10% mortality was observed at 10 mg/L and 100% in the three highest test item concentrations of 22, 50 and 100 mg/L, respectively. At the concentration of 10 mg N-methyl-bentazone/L surviving fish showed sub-lethal effects (apathy).

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) for N-methyl-bentazone (metabolite of bentazone) was determined to be 8.56 mg/L, based on mean measured concentrations. The NOEC (96 h) was 3.9 mg/L (mean measured).

I. MATERIAL AND METHODS

Test item:	N-methyl-bentazone (BH 351-N-ME, metabolite of BAS 351 H, Reg. No. 79 520), batch no. 2235-09, purity: 99.8%.
Test species:	Rainbow trout (*Oncorhynchus mykiss*), approx. 4 months old; mean body length 5.0 cm (4.6 - 5.6 cm); mean body weight 1.13 g (0.76 - 1.58 g); supplied by Forellenzucht Trostadt GbR, Trostadt, Germany.
Test design:	Static system (96 hours); 10 fish per aquarium (loading 0.2 g fish/L), 2 replicates per concentration; assessment of mortality and symptoms of toxicity within 1 hour after start of exposure and 4, 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Control, 5, 10, 22, 50 and 100 mg N-methyl-bentazone/L (nominal).
Test conditions:	Glass aquaria with stainless steel frames (60 x 35 x 40 cm), test volume 50 L, non-chlorinated, filtered tap water; temperature: 11 °C - 13 °C; pH 8.2 - 8.4; oxygen content: 8.1 mg/L - 10.6 mg/L; total hardness: about 250 mg $CaCO_3$/L; conductivity: 550 µS/cm^2; photoperiod 16 h light : 8 h dark; light intensity: 75 lux - 236 lux; no aeration, no feeding.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.
Statistics:	Descriptive statistics; probit analysis for calculation of LC_{50}.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at test initiation and at the end of the test. Measured concentrations for N-methyl-bentazone ranged from 75.3% to 87.7% of nominal at test initiation for the concentrations 5 - 50 mg/L. The recoveries for the nominal content of 100 mg/L were analyzed to be 53.6% - 108.0%. The concentration is above the LC_{100} and therefore not relevant for the determination of the effect concentration. Due to the biological results only the samples with the nominal contents of 5 - 22 mg/L were analyzed at test termination. Recoveries were in the range between 73.5% - 83.7% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure no mortality and toxic effects were observed in the control and at the concentrations of 5 mg N-methyl-bentazone/L, whereas 10% mortality was observed at 10 mg/L and 100% in the three highest test item concentrations of 22, 50 and 100 mg/L, respectively. At the concentration of 10 mg N-methyl-bentazone/L surviving fish showed sub-lethal effects (apathy). The results are summarized in Table 8.2/2.

Table 8.2/2 Acute toxicity (96 h) of N-methyl-bentazone (metabolite of bentazone) to rainbow trout (*Oncorhynchus mykiss*)

Concentration [mg/L] nominal	**Control**	**5**	**10**	**22**	**50**	**100**
Mortality [%]	0	0	10	100	100	100
Symptoms *	none	none	A	n.d.	n.d.	n.d.
Endpoints [mg N-methyl-bentazone/L] (mean measured)						
LC_{50} (96 h)	8.56					
NOEC (96 h)	3.9					

* Symptoms: A = apathy
n.d. = not determined; all fish dead

III. CONCLUSION

In a static acute toxicity study with rainbow trout the LC_{50} (96 h) for N-methyl-bentazone (metabolite of bentazone) was determined to be 8.56 mg/L, based on mean measured concentrations. The NOEC (96 h) was 3.9 mg/L (mean measured).

8.2.2 Chronic toxicity to fish

8.2.3 Chronic toxicity (28 day exposure) to juvenile fish

A 28-day test on *Oncorhynchus mykiss* was performed with bentazone (tested with the solo-formulation BAS 351 32 H (= Basagran; containing 48% bentazone); [redacted] 1988, BASF DocID 1988/10056) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

In the 28-day flow-through toxicity study with rainbow trout the NOEC for bentazone (tested as BAS 351 32 H) was determined to be 48 mg a.s./L (nominal).

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996) and in the EC Review Report (7585/VI/97-final, November 2000) for bentazone.

Additionally, a 28-day chronic toxicity study on *Oncorhynchus mykiss* was performed with the bentazone metabolite N-methyl-bentazone (= M351H009). It is reported here as it contains relevant information and was only recently finalized and not submitted yet in an Annex II dossier.

Reference number:	II A 8.2.3/1
Report:	[redacted] 2006(b) Reg.No. 79520 (metabolite of BAS 351 H) - Juvenile growth test in rainbow trout (Oncorhynchus mykiss) in a flow through system (28 days) [redacted] unpublished BASF DocID 2006/1008192
Guidelines:	OECD 215 (2000)
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 28-day flow-through chronic toxicity laboratory study, rainbow trout were exposed to nominal concentrations of 0.075, 0.23, 0.75, 2.3 and 7.5 mg N-methyl-bentazone/L (= M351H009) and a water control in groups of 20 animals in glass aquaria containing 40 L water. Fish were observed for survival and symptoms of toxicity once per day until test termination 28 days after start of exposure. Mean lengths and mean weights of surviving fish were recorded after 14 days of exposure and after 28 days.

The biological results are based on nominal concentrations. In addition results based on the mean measured concentrations are reported. After 28 days 11% mortality was observed in the test group 0.23 mg/L. This level is acceptable for control mortalities and thus not considered to reflect a clear substance related effect. Furthermore, no abnormalities or abnormal behaviour were observed. There was no effect on growth either. In the test groups 0.75 mg/L and 2.3 mg/L statistically significant mortality of 70% and 80% was observed. In the test group 2.3 mg/L from day 15 and in the test group 0.75 mg/L from day 20 onwards reduced food consumption was observed. In the 2.3 mg/L group body weight and length after 28 days was statistically significantly reduced. In the test group 7.5 mg/L mortality was 40%. The mortality was lower than in the lower concentrations and does therefore not follow a monotonous concentration effect relationship. A reduced food uptake was observed over the whole exposure period and from day 18 onwards tumbling of some individuals was observed. Mortality, body length and weight were statistically significantly reduced.

In a 28-d flow-through chronic toxicity study with rainbow trout (*O. mykiss*) the NOEC of N-methyl-bentazone (metabolite of bentazone) was 0.23 mg/L (nominal).

I. MATERIAL AND METHODS

Test item:	N-methyl-bentazone (BH 351-N-ME, metabolite of BAS 351 H, Reg. No. 79 520), batch no. L75-7, purity: 99.6%.
Test species:	Rainbow trout (*Oncorhynchus mykiss*), mean body length 5.0 - 5.7 cm, mean body weight 1.4 - 1.8 g; supplied by Forellenzucht Trostadt GbR, Trostadt, Germany.
Test design:	Flow-through system (28 days); 5 test concentrations plus control; 20 fish per aquarium and per concentration; daily assessment of mortality and symptoms of toxicity; assessment of mean lengths and mean weights of surviving fish after 14 days and at test termination after 28 days.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Dilution water control, 0.075, 0.23, 0.75, 2.3 and 7.5 mg N-methyl-bentazone/L (nominal).
Test conditions:	Glass aquaria (59 x 28 x 30 cm), test volume: 40 L, purified non-chlorinated drinking water; temperature: 14 °C; pH 7.7 - 7.8; oxygen content: 6.9 mg/L - 9.4 mg/L; total hardness: approx. 140 mg $CaCO_3$/L; flow rates of test solutions: 8.33 L/hour/test vessel, photoperiod 16 h light : 8 h dark; no aeration.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with UV-detection.
Statistics:	Descriptive statistics; Fisher's exact test for mortality data, Dunnett's test for body weight and length ($p \leq 0.01$).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration on day 0, 4, 11, 14, 20, 25, for the concentration of 0.075 mg/L additionally on days 6 and 26 and for the concentration of 7.5 mg/L on day 12. The analyzed contents of N-methyl-bentazone ranged from 89.5% to 102.7% of nominal at test initiation and from 100.1% to 119.9% of nominal at test termination, except for the lowest concentration group of 0.075 mg/L where mean recovery was 134.7% at test termination. The mean recovery during the whole study was ± 20% of the nominal concentrations, except for an interruption of exposure in the 7.5 mg/L group for 24 h and two transient occasions of the lowest concentration of 0.075 mg/L. The maintenance of the concentrations was considered to be sufficient for an evaluation of the study results. Therefore, the following biological results are based on nominal concentrations. In addition results based on the mean measured concentrations are reported.

After 28 days 11% mortality was observed in the test group 0.23 mg/L. This level is acceptable for control mortalities and thus not considered to reflect a clear substance related effect. Furthermore, no abnormalities or abnormal behavior were observed. There was no effect on growth either. In the test groups 0.75 mg/L and 2.3 mg/L statistically significant mortality of 70% and 80% was observed (Fisher's exact test, $p \leq 0.01$). In the test group 2.3 mg/L from day 15 and in the test group 0.75 mg/L from day 20 onwards reduced food consumption was observed. In the 2.3 mg/L group body weight and length after 28 days was statistically significantly reduced (Dunnett's test, $p \leq 0.01$). In the test group 7.5 mg/L mortality was 40%. The mortality was lower than in the lower concentrations and does therefore not follow a monotonous concentration effect relationship. A reduced food uptake was observed over the whole exposure period and from day 18 onwards tumbling of some individuals was observed. Mortality, body length and weight were statistically significantly reduced (Fisher's exact test, Dunnett's test, $p \leq 0.01$). The results are summarized in Table 8.2/3.

Table 8.2/3 Chronic toxicity (28 d) of N-methyl-bentazone (metabolite of bentazone) on rainbow trout (*O. mykiss*)

Concentration [mg/L] (nominal)	**Control**	**0.075**	**0.23**	**0.75**	**2.3**	**7.5**
Mortality [%]	0	0 #	11 #	70 **	80 **	40 **
Mean body length on day 0 [cm]	5.270	5.375	5.290	5.385	5.245	5.360
Mean body length on day 14 [cm]	6.325	6.450	6.420	6.432	6.205	5.537*
Mean body length on day 28 [cm]	7.880	8.005	7.947	7.567	6.975*	5.775*
Mean wet weight on day 0 [g]	1.535	1.570	1.495	1.535	1.530	1.570
Mean wet weight on day 14 [g]	2.970	2.980	2.845	2.953	2.726	1.747*
Mean wet weight on day 28 [g]	5.275	5.500	5.335	5.000	3.825 *	1.592 *
	Endpoints [mg N-methyl-bentazone/L] nominal					
$NOEC_{overall}$ (28 d)	0.23 (mean measured 0.228)					
$LOEC_{overall}$ (28 d)	0.75 (mean measured 0.735)					

* Statistically significant differences compared to the control (Dunnett's test, $p \leq 0.01$)
** Statistically significant differences compared to the control (Fisher's exact test, $p \leq 0.01$)
# Between study day 14 and end of exposure one fish jumped out of the aquarium.

III. CONCLUSION

In a 28-d flow-through chronic toxicity study with rainbow trout (*O. mykiss*) the NOEC of N-methyl-bentazone (metabolite of bentazone) was 0.23 mg/L (nominal).

8.2.4 Fish early life stage toxicity test

An early life-stage toxicity study on *Pimephales promelas* has been performed with the Na-salt of bentazone as required for US registration purposes. It is reported here as it contains relevant information and was only recently finalized and not submitted yet in an Annex II dossier.

Report:	II A 8.2.4/1 [redacted] 2011(b) Reg.No. 88691 (Bentazone-sodium, BAS 351 H-Na) - Early-life-stage toxicity test on the fathead minnow (Pimephales promelas) in a flow through system BASF DocID 2011/1256797
Guidelines:	OECD 210; EPA 72-4 (a); EPA 850.1400; EPA 540/9-86-138
Deviations:	none
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

The chronic toxicity of bentazone-Na to fathead minnow (*Pimephales promelas*) was evaluated in a 35-day early life-stage test under flow-through conditions. Embryos were exposed to a dilution water control and to bentazone-Na at a single concentration of 10 mg a.s./L (nominal). Hatchability, pre- and post-hatch survival rate, time to hatch and swim-up, signs of toxicity and growth parameters of fathead minnow embryos and larvae were assessed throughout the study.

The biological results are based on nominal concentrations. No statistically significant adverse effects on hatching success, stage -specific and overall survival, toxic signs, time spans to hatch and swim-up and growth rates compared to the control were observed at up to and including the highest tested concentration of 10 mg bentazone-Na/L.

In an early life stage study with fathead minnow (*Pimephales promelas*), the overall NOEC (35 d) for bentazone-Na was determined to be ≥ 10 mg a.s./L based on nominal concentrations.

I. MATERIAL AND METHODS

Test item:	Bentazone-Na (Bentazone-sodium, BAS 351 H-Na, Reg. No. 88 691), batch no. COD-001417, purity: 91.9% (tolerance ± 1.0%).
Test species:	Fathead minnow (*Pimephales promelas*); embryos (fertilized eggs, less than 3.5 hours old), source: parental hatched and were raised in-house.
Test design:	Flow-through system (35 d); 1 test item concentration plus a dilution water control, 4 replicates per treatment with 25 embryos in each (maximum loading at the end of the test: 0.12 g fish/L/day). Fertilized eggs (embryos) and larvae were exposed in cylindrical glass vessels and were transferred into stainless steel aquaria on day 15. The test solution flowed continuously from the mixing tank into an "udder", which splitted the test solution into 4 equal parts for the 4 replicate test aquaria. On day 35 fish were sacrificed and the body length and weight of surviving individuals were determined. Daily assessment of hatch, swim-up, survival, signs of toxicity and abnormal behaviour.
Endpoints:	NOEC values based on hatching success, stage -specific and overall survival, toxic signs, overall growth and time spans to hatch and swim-up.
Test concentrations:	Control (dilution water) and 10 mg bentazone-Na/L (nominal).
Test conditions:	Test vessels: cylindrical glass vessels, water volume: 1.7 L (until day 15); stainless steel aquaria (29 x 21 x 22 cm), water volume: 9.0 L (from day 15 until test end); dilution water: non-chlorinated, filtered tap water (diluted with deionized water); temperature 24.7 °C - 26.1 °C; pH 7.7 - 8.1; oxygen content: 5.6 mg/L - 8.0 mg/L; total hardness: approx. 95 - 104 mg $CaCO_3$/L; conductivity: 238 µS - 257 µS; acid capacity: 2.32 - 2.44 mmol/L; photoperiod: 16 hours light : 8 hours dark; light intensity: 127 lux - 249 lux; flow rates: 150 mL/minute/treatment group, resulting in 2.25 L/hour/test vessel (providing a 6-fold exchange rate of the water volume in each larger test vessel (9 L) every 24 hours); feeding: combination of live brine shrimp nauplii (*Artemia* sp.) and fine milled commercial fish diet ("Tetramin"), three times daily on workdays and twice daily on non-working days; no aeration until day 18; slight aeration from day 18 on due decreased oxygen content.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.
Statistics:	Descriptive statistics; Student`s test for weight and length data; one-sided Fisher's exact test for survival data, one-sided Wilcoxon-test for variability between replicates.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in all test item concentrations at the beginning of the test and at regular intervals until day 35 (generally before replacing the stock solutions). Mean measured concentrations of bentazone-Na ranged from 93.6% to 101.9% of nominal concentrations in the test item treatments and were within the range ±20% of nominal during the whole experiment. As measured concentrations confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: No statistically significant adverse effects on hatching success, stage -specific and overall survival, toxic signs, time spans to hatch and swim-up and growth rates compared to the control were observed at the tested concentration of 10 mg bentazone-Na/L (Student`s test for growth data; Fisher's exact test and Wilcoxon test for survival data). The results are summarized in Table 8.2/4.

Table 8.2/4 Chronic toxicity of bentazone-Na to fathead minnow (*Pimephales promelas*) in a fish early life stage test (35 d)

Concentration (nominal) [mg a.s./L]	**Control**	**10**
Hatching success [%]	97	98
Survival of larvae from hatch until end of swim-up (day 6) [%]	100	99
Survival of young fish from end of swim-up until test end (day 6 to 35) [%]	94	91
Survival from day 0 to test termination (day 35) [%]	91	88
Start of hatch [day] End of hatch [day]	3 5	3 5
Start of swim-up End of swim-up	4 6	4 6
Symptoms	none	none
Mean weight (35 d) [mg]	242.3	251.6
% of control	100	104
Mean length (35 d) [cm]	2.6	2.7 *
% of control	100	103
	Endpoint [mg a.s./L] (nominal)	
$NOEC_{overall}$ (35 d)	≥ 10	

* Statistically significantly greater compared to control data (Student´s t-test, $p < 0.05$); however, this is not an adverse or treatment-related effect. No other statistically significant differences compared to the control were observed for growth data (Student´s t-test) and survival data (Fisher's exact test).

III. CONCLUSION

In an early life stage study with fathead minnow (*Pimephales promelas*), the overall NOEC (35 d) for bentazone-Na was determined to be ≥ 10 mg a.s./L based on nominal concentrations.

8.2.5 Fish life cycle test

A fish life cycle test is not required, since a fish early life stage toxicity test is available (see M-II Point 8.2.4).

8.2.6 Bioconcentration potential in fish

8.2.6.1 Bioconcentration potential of the active substance in fish

Reference number: II A 8.2.6.1/1
Report: [redacted] 1992(a)
Bioaccumulation and metabolism 14C-Bentazon in bluegill sunfish
[redacted]
unpublished
BASF DocID 1992/5204
Guidelines: EPA 165-4
GLP: Yes
(laboratory certified by United States Environmental Protection Agency)

Executive Summary

The bioaccumulation and metabolism of ^{14}C-bentazone has been investigated in bluegill sunfish in a dynamic flow-through system. Fish were exposed to the test item at a concentration of 4.9 ppm for a 28-day uptake phase followed by a depuration period of 17 days (depuration phase). A control experiment with fish exposed to diluent water only was maintained in parallel. Uptake, depuration, and bioconcentration of bentazone were determined in whole body, edible and non-edible tissue. Water and fish samples were taken during the study and analyzed radiometrically for total ^{14}C-test material concentrations, then converted to bentazone (radiolabeled plus non-radiolabeled bentazone) with the test material stock solution's specific activity. Additional treated fish and exposure water samples were taken for metabolite identification.

During the exposure phase no mortalities were observed in the control group and in the bentazone treatment group. One control fish died on the last day of the depuration phase. Fish showed no visible signs of stress during the uptake or depuration phase of the study and accepted food readily each day.
Radioactive residues, accumulated very slightly in bluegill sunfish. Edible tissues accounted for approximately 18% of the sum of edible and non-edible radioactive residues on day 28. The bioconcentration factors (BCFs) for whole body, edible and non-edible tissues were, 1.4, 0.4 and 2.3, respectively. The elimination half-life for bentazone was 23.1 days for edible tissue and 13.9 days for non-edible tissue and whole fish. Steady-state conditions between tissue concentration and water concentration of bentazone were attained in all tissue groups by day 7 of the exposure phase.

The range of total residues for non-edible tissues was 10.7 to 12.7 ppm for the accumulation phase and 5 ppm for the depuration phase. For edible tissues, the total residue levels were 2.0 ppm for the accumulation phase and 1.4 ppm for the depuration phase. For non-edible tissues, the marc (methanol non-extractable residues) contained about 3.5 ppm for the accumulation phase and 1.6 ppm for the depuration phase. For edible tissues the marc held 0.9 ppm in the accumulation phase, and 0.7 ppm in the depuration phase. Acid digestion of the marc afforded a DCM extract that held much less than a microgram of material in most cases. Even after acid methanolysis, the majority of radioactivity remained with the marc which indicates that these residues would not be available for further metabolism.

Only the parent bentazone could be clearly identified from this metabolism study. However, there was enough radioactivity to show at least nine distinct metabolites which confirmed the previous conclusion that the residues are a complex mixture of components. When homogeneous spots were isolated by TLC, the mass spectral total ion chromatograms showed them to include complex mixtures of endogenous compounds. As a result, the observed data indicate that no individual residue, other than bentazone, was present in sufficient concentration for identification.

In a flow-through bioconcentration study with bluegill sunfish with an uptake period of 28 days and a depuration period of 17 days, the BCF for bentazone was determined to be 1.4 (whole fish) at steady state. The low bioconcentration factors and the rapid elimination of radioactive residues demonstrate a low bioconcentration potential for bentazone.

I. MATERIAL AND METHODS

Test item: Bentazone (BAS 351 H, Reg. No. 51 929, ^{14}C), radiolabeled test substance: phenyl-U-^{14}C label, lot no. 298-6, radiochemical purity: 97.5% (by radio-TLC) and 97% (by HPLC/LSC), specific activity 33.95 mCi/mmol (3.123 x 10^5 dpm/µg); unlabeled test substance: technical grade sodium bentazone (aqueous solution, 48.5% bentazone), lot no. 68-901102, purity: > 99% (HPLC).

Test species: Bluegill sunfish (*Lepomis macrochirus*), mean body weight: 0.95 g (0.64 - 1.32 g), mean body length: 34.2 mm (33 - 36 mm), source: Osage catfisheries Inc., Osage Beach (MO), USA.

Test design: Flow through system (28 d uptake, 17 d depuration); one test item concentrations plus a dilution water control, 94 fish per group. Fish maintained in glass aquaria. The ^{14}C-labeled test material was delivered at a rate of 73 cycles/day via a flow-through diluter system to the test chamber. For the depuration phase fish were transferred to glass aquaria containing dilution water only. Four fish were sampled from the test item treatment group on days 1, 3, 7, 10, 14, 21, 24, and 28 of the uptake period, and on days 1, 3, 7, 10, and 14 of the depuration period. Four to six control fish were sampled on days 0, 21 and 28 of the exposure phase. Six fish were collected from the test item treatment group on day 45. Additional fish exposed to the test item were sampled on days 10 and 28 of the exposure phase and on day 7 of the depuration phase for metabolite identification analyses. On days 21 and 28 additional fish were taken from the control. After sampling, fish were sacrificed, blotted dry and the body length and weight were determined. Two fish were used for determination of whole body ^{14}C-bentazone activity. Two further fish were dissected and pooled into edible (body, muscle, skin, and skeleton) and non-edible (fins, head, and viscera) tissues.
Triplicate water samples from the control group and the test item treatment group were collected daily during the uptake period and on day 1 of the depuration period. Additional samples of 1 L test solution were sampled from the test item treatment group on days 0, 10 and 28 of the exposure phase and on day 7 of the depuration phase for metabolite analyses. 1 L samples were collected from the control group on days 21 and 28. Three to five mL samples of new stock solutions were taken on days 0, 7, 14 and 21.

Endpoints: Bioaccumulation potential (bioconcentration factors BCF), uptake rate, depuration rate, depuration half-life (DT_{50}). Metabolism.

Test concentrations: Control (dilution water), 4.9 ppm

Test conditions: Test vessels: 60 L glass aquaria; water volume: 44 L; flow-through system; dilution water: mixture of activated carbon and iron-filtered well water and reverse osmosis treated well water; loading (day 0): 0.49 g fish/L/day; flow rates: 182 L/day/test vessel (0.126L/min/vessel); temperature 20.8 °C - 23.2 °C; pH 6.8 - 7.4; oxygen content 6.3 - 8.6 mg/L; hardness: approx. 65 mg $CaCO_3$/L; lightning: fluorescent light bulbs; photoperiod: 16 hours light : 8 hours dark; feeding: frozen brine shrimp (2% of body weight).

Analytics: Tank water metabolites/degradates were quantitated and the purity of the ^{14}C-bentazone and technical grade bentazone was analyzed for chemical purity by HPLC. Radiometric assays were performed by liquid scintillation counting (LSC). Radioactivity in soild and some liquid samples was determined by LSC after combustion. For analysis of residues from fish tissues several extraction and fractionation steps were performed; examination by radio-TLC. Analysis of radioactive residues in stock solutions and exposure tank waters after extraction was performed using radio-TLC. Mass spectral analysis (GC/MS) was conducted for identification of isolated metabolites.

Calculations: BCFs for each tissue type was calculated by dividing the apparent steady-state average tissue residue concentration by the average water concentration. In addition, a nonlinear regression analysis was used to determine the BCFs for all three tissue groups.

II. RESULTS AND DISCUSSION

Fish behavior and mortality: During the exposure phase no mortalities were observed in the control group and in the bentazone treatment group. One control fish died on the last day of the depuration phase. Fish showed no visible signs of stress during the uptake or depuration phase of the study and accepted food readily each day.

Total radioactive residues: Bioconcentration values for the tissues were: edible tissue, 0.4; non-edible tissue, 2.2 (by nonlinear estimation) and 2.3 (by steady-state estimation); and whole body, 1.4. Depuration data indicated that elimination of ^{14}C-bentazone in edible tissue was slower than in non-edible tissue: the K_d values were 0.03 d^{-1} for edible tissue and 0.05 d^{-1} for non-edible tissue and whole fish. Half-life ($t_{1/2}$) values were 23.1 days for edible tissue and 13.9 days for non-edible tissue and whole fish. Steady-state conditions between tissue concentration and water concentration of bentazone were attained in all tissue groups by day 7 of the exposure phase. The BCF values for the bentazone residues in each tissue group are given in see Table 8.2/5.

Table 8.2/5 Bioconcentration factor (BCF) for each tissue group

Analysis	Tissue fraction		
	Edible	Non-edible	Whole body
Nonlinear estimation [1)]	0.4	2.2	1.4
Steady-state estimation [2)]	0.4	2.3	1.4

1) BCF = k_u/k_d, with k_u and k_d estimated from nonlinear regression
2) BCF = C_{ss}/C_w, where C was calculated as the mean ^{14}C-bentazone in each tissue group on sample days 7 through 28.

The k_d and $t_{1/2}$ values for ^{14}C-bentazone in the whole body, non-edible, and edible tissue are given in Table 8.2/6.

Table 8.2/6 Elimination of bentazone residues from fish tissue groups

Parameter	Tissue fraction		
	Edible	Non-edible	Whole body
Depuration rat constant k_d [$days^{-1}$]	0.03	0.05	0.05
R-squared *	0.49	0.86	0.87
Depuration half-life DT_{50} ($t_{1/2}$) [days]	23.1	13.9	13.9

* R-squared coefficient of determination for linear regression analysis.

Analyses of exposure water: The exposure tank water was analyzed during the accumulation phase of the study at 0-, 10- and 28-day periods. The only extractable product observed in the tank water was the parent material which represented 100% of the region of quantitation. Therefore, these TLC chromatograms verify that ^{14}C-bentazone was stable in the treating solution throughout the 28-day treating period. The average specific activity in the treating solution was 2,619 dpm/mL and the average specific activity of ^{14}C-bentazone was 595 dpm/g.

Analyses of fish tissues: For the non-edible tissues, the residues (% TRR, ppm) observed in the methanol extract were: 10-day accumulation phase (65.3%, 7.0), 28-day accumulation phase (72.9%, 9.3) and 7-day depuration phase (67.9%, 3.4). Therefore, the residues (% TRR, ppm) remaining in the marc from non-edible tissues were: 10-day (34.7%, 3.7), 28-day (27.1%, 3.4) and 7-day (32.1%, 1.6). For the edible tissue for the 7-day depuration phase, the residues (% TRR, ppm) were determined for the methanol extract (53%, 0.7) and for the insoluble marc (47%, 0.7). For both edible and non-edible tissues, the methanol extracts were fractionated into DCM and ethyl acetate phases to isolate metabolites according to solubilities. After TLC purification, the isolated metabolites were examined by mass spectral analysis for identification. There were at least four distinct neutral and/or basic residues in the DCM phase and six distinct acidic residues in the ethyl acetate phase. These residues were generally in the 10% TRR range or less. Bentazone, as its N-methylated derivative, was the only residue that could be identified. All other residues were isolated in such small quantities or isolated fractions were of such low concentration that suitable purity could not be achieved for mass spectral analysis.

III. CONCLUSION

The uptake has reached steady-state conditions within 7 days. Calculation of the bioconcentration factor BCF based on measured radioactivity in tank water and fish at steady-state resulted in values of 1.4 for whole fish. With a BCF of less than one, residues in the edible tissue were extremely low; thus, no metabolic byproduct from edible tissue could be identified in this study. Only the parent compound bentazone could be clearly identified from this metabolism study. However, there was enough radioactivity to show at least nine distinct metabolites indicating that the residues are a complex mixture of components. As a result, the observed data indicate that no individual residue, other than bentazone, was present in sufficient concentration for identification.

Considering the low bioconcentration factors and the rapid elimination of radioactive residues, the bioconcentration potential of bentazone in fish can be classified as low.

8.2.6.2 Bioconcentration potential of the metabolites, degr. & react. products

No separate study on bioconcentration potential of metabolites was conducted. The observed data of the study under chapter 8.2.6.1 indicate that no individual residue, other than bentazone, was present in sufficient concentration for identification.

8.2.7 Aquatic bioavailability/ biomagnification / depuration

The bioconcentration potential is very low and depuration is fast (see 8.2.6.1). Thus, there is no risk of biomagnification and no further specific studies are required.

Summary of results

The effects of bentazone and its metabolite N-methyl-bentazone (= M351H009) on fish are summarized in Table 8.2/7

Additionally, a BCF study with *L. macrochirus* was conducted under flow-through conditions and resulted in a BCF of 1.4 (whole fish). Rapid elimination of the test item was observed with a depuration half life of 13.9 days (whole fish).

Table 8.2/7 Summary of results of bentazone and metabolite on aquatic invertebrates

Test species	Test system	Result [mg/L]		Reference
		LC_{50}	NOEC	
Bentazone				
O. mykiss	Static 96 h	> 100	50	[redacted], 1987/0428
P. promelas #	Static 96 h	> 120	≥ 120	[redacted] 2011/1173854
L. macrochirus	Static 96 h	> 100	100	[redacted] 1986/9005
C. carpio #	Static 96 h	> 1000	560	[redacted] 1983/10048
O. mykiss *	Flow-through, 28 d	--	48	[redacted] 1988/10056
P. promelas #	ELS, flow-through, 35 d	--	≥ 10	[redacted] 2011/1256797
Lysimeter leachate +				
Brachydanio rerio	Static 96 h	no effect	--	[redacted] 1994/11094
N-methyl-bentazone (= M351H009, Reg. No. 79 520)				
O. mykiss	Static 96 h	8.56	3.9	[redacted] 2004/1025175
O. mykiss *	Flow-through, 28 d	--	0.23	[redacted] 2006/1008192

Study was conducted with the Na-salt of bentazone.
* Study was conducted with the solo formulation BAS 351 32 H (Basagran), containing 48% bentazone.
\+ Study was conducted with leachate waters from BAS 351 32 H treated lysimeters.

8.3 Toxicity to aquatic species other than fish, aquatic field tests

8.3.1 Acute toxicity to aquatic invertebrates

8.3.1.1 Acute toxicity (24 and 48 hour) for Daphnia preferably (Daphnia magna)

An acute toxicity study on *Daphnia magna* was performed with bentazone (Bias, 1986, BASF DocID 1986/9009) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

In the static acute toxicity study with *Daphnia magna* the EC_{50} (48 h) of bentazone was 125 mg a.s./L based on nominal concentrations. The EC_0 (48 h) was determined to be 62.5 mg a.s./L (nominal).

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996). For verification reasons a new study on *D. magna* by Jatzek (DocID 2003/1004524, see below) was performed in 2003. These results are considered relevant for acute risk assessment on aquatic invertebrates.

Additionally, an acute toxicity study on *Daphnia magna* was performed with bentazone (tested with the lysimeter leachate of the formulation BAS 351 32 H; Dohmen, 1994, BASF DocID 1994/11094) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

None of the leachate waters caused any immobilization or had any other observable effects on the daphnids. Hence, there is no indication that these residues, which would normally be much diluted before any exposure to aquatic organisms occurs, are of any ecotoxicological concern to the sensitive test organism *Daphnia magna*.

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996).

Furthermore, acute toxicity studies on *Daphnia magna* have been performed with bentazone and the bentazone metabolites N-methyl-bentazone (=M351H009), Peak B (=M351H023) and Peak C (=M351H024). They are reported here as they contain relevant information and were only recently finalized and not submitted yet in an Annex II dossier.

Reference number:	II A 8.3.1.1/1
Report:	Jatzek H.J. 2003(b) BAS 351 H (Bentazone) - Determination of the acute effect on the swimming ability of the water flea Daphnia magna STRAUS BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. unpublished BASF DocID 2003/1004524
Guidelines:	EEC 92/32 A V C 2; OECD 202 Part I (1984); EPA 850.1010; ISO 6341; ISO/DIS 10706
GLP:	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a static acute toxicity laboratory study, water flea neonates were exposed to bentazone at nominal concentrations of 12.5, 25, 50 and 100 mg bentazone/L in 4 replicates per concentration containing 5 daphnids each. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.

The biological results are based on nominal concentrations of the test item. No immobility occurred in the control and any test concentration after 24 h. After 48 hours 5% immobility was observed in the control and the test concentration of 12.5 mg/L.

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of bentazone was determined to be > 100 mg/L based on nominal concentrations. The NOEC was ≥ 100 mg/L (nominal).

I. MATERIAL AND METHODS

Test item: Bentazone (BAS 351 H, Reg. no. 51 929), batch no. 14-7887, purity: 98.4%.

Test species: Water flea (*Daphnia magna* STRAUS), neonates from in-house culture, > 2 < 24 hours old at test initiation.

Test design: Static system (48 hours), 4 test concentrations and a control, 4 replicates with 5 daphnids in each; assessment of immobility (and other effects) after 24 and 48 hours.

Endpoints: EC_{50} and NOEC based on immobility of daphnids.

Test concentrations: Control, 12.5, 25, 50, 100 mg bentazone/L (nominal).

Test conditions: Glass vessels, test volume 50 mL, dilution water: Elendt "M4" medium; total hardness: 2.44 mmol/L (at test initiation), conductivity: 608 µS/cm (at test initiation), temperature: 18.8 °C - 19.5 °C; pH value: 6.8 - 8.1; oxygen content: 8.5 mg/L - 9.2 mg/L; photoperiod: 16 hours light : 8 hours dark; no feeding and no aeration.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. Measured values for bentazone ranged from 103% to 104% of nominal concentrations at test initiation and from 102% to 103% at test termination. As analytical data confirmed correct application of the test item the following biological results are based on nominal concentrations.

Biological results: No immobility occurred in the control and any test concentration after 24 h. After 48 hours 5% immobility was observed in the control and the test concentration of 12.5 mg/L. The results are summarized in Table 8.3/1.

Table 8.3/1 Immobility of *D. magna* resulting from exposure to bentazone

Concentration [mg/L] nominal	**Control**	**12.5**	**25**	**50**	**100**
Immobile (24 h) [%]	0	0	0	0	0
Immobile (48 h) [%]	5	5	0	0	0
Endpoints [mg bentazone/L] (nominal)					
EC_{50} (48 h)	> 100				
NOEC (48 h)	≥ 100				

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of bentazone was determined to be > 100 mg/L based on nominal concentrations. The NOEC was ≥ 100 mg/L (nominal).

Reference number:	II A 8.3.1.1/2
Report:	Funk M. 2004(b) Effect of BH 351-N-Me (Reg.No. 79 520; metabolite of BAS 351 H) on the immobility of Daphnia magna STRAUS in a 48 hours static, acute toxicity test BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. unpublished BASF DocID 2004/1003973
Guidelines:	OECD 202; EEC 79/831 A V C 2; EPA 72-2; EPA 850.1010
GLP:	Yes (laboratory certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany Fed.Rep.)

Executive Summary

In a static acute toxicity laboratory study, water flea neonates were exposed to N-methyl-bentazone (metabolite of bentazone, M351H009) at nominal concentrations of 10, 16, 32, 56 and 100 mg N-methyl-bentazone/L in 4 replicates per concentration containing 5 daphnids each. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.

The biological results are based on mean measured concentrations of the test item. No immobility occurred at 15.3 mg N-methyl-bentazone/L. Immobility of the daphnids was observed at the test concentration of 31.3 mg/L and higher after 24 and 48 hours.

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of N-methyl-bentazone (metabolite of bentazone) was determined to be 26.5 mg/L based on mean measured concentrations. The NOEC was 15.3 mg/L (mean measured).

I. MATERIAL AND METHODS

Test item:	N-methyl-bentazone (BH 351-N-ME, metabolite of bentazone, Reg. No. 79 520), batch no. 2235-09, purity: 99.8%.
Test species:	Water flea (*Daphnia magna* STRAUS), neonates from in-house culture, < 24 hours old at test initiation.
Test design:	Static system (48 hours), 5 test concentrations plus 2 controls, 4 replicates with 5 daphnids in each; assessment of immobility (and other effects) after 24 and 48 hours.
Endpoints:	EC_{50} and NOEC based on immobility of daphnids.
Test concentrations:	Control, Control + Chremophor (0.1 mL/1000 mL "M4" medium), 10, 16, 32, 56 and 100 mg N-methyl-bentazone/L (nominal).
Test conditions:	Glass vessels, test volume 50 mL, dilution water: Elendt "M4" medium; , total hardness: 2.41 mmol/L (at test initiation), conductivity: 609 µS/cm (at test initiation), temperature: 20.4 °C - 21.3 °C; pH value: 7.77 - 8.01; oxygen content: 8.42 mg/L - 9.50 mg/L; photoperiod: 16 hours light : 8 hours dark; light intensity: < 1500 lux, no feeding and no aeration.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.
Statistics:	Descriptive statistics, log-log analysis and Spearman-Karber for determination of EC_{50} value.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. Measured values for N-methyl-bentazone ranged from 93.3% to 98.5% of nominal concentrations at test initiation and from 97.3% to 99.7% at test termination. The analytical data confirmed correct application of the test item. The following biological results are based on mean measured concentrations.

Biological results: No immobility occurred at 15.3 mg N-methyl-bentazone/L. Immobility of the daphnids was observed at the test concentration of 31.3 mg/L and higher after 24 and 48 hours. The results are summarized in Table 8.3/2.

Table 8.3/2 Immobility of *D. magna* resulting from exposure to N-methyl-bentazone (metabolite of bentazone)

Concentration [mg/L] nominal	**Control**	**Control + Chremophor**	**10**	**16**	**32**	**56**	**100**
Concentration [mg/L] mean measured	**Control**	**Control + Chremophor**	**9.8**	**15.3**	**31.3**	**54.5**	**97.9**
Immobile (24 h) [%]	0	0	0	0	15	15	100
Immobile (48 h) [%]	0	0	0	0	70	100	100
Endpoints [mg N-methyl-bentazone/L] (mean measured)							
EC_{50} (48 h)	26.5 (95% CL: 23.31- 30.08)						
NOEC (48 h)	15.3 (95% CL: 55.27 - 73.20)						

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of N-methyl-bentazone (metabolite of bentazone) was determined to be 26.5 mg/L based on mean measured concentrations. The NOEC was 15.3 mg/L (mean measured).

Reference number:	II A 8.3.1.1/3
Report:	Dorner S. 2011(a) Acute toxicity of Reg.No. 5831080 (Peak B, metabolite of BAS 351 H) to Daphnia magna STRAUS in a 48 hour static test BASF SE; Limburgerhof; Germany Fed.Rep. unpublished BASF DocID 2011/1163524
Guidelines:	OECD 202; EPA 850.1010
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a static acute toxicity laboratory study, water flea neonates were exposed to Peak B (metabolite of bentazone, M351H023) at one concentration of 100 mg Peak B/L in 4 replicates per concentration containing 5 daphnids each. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.

The biological results are based on nominal concentrations of the test item. No immobility occurred at 100 mg Peak B/L and 5% immobility was observed in the control.

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of Peak B (metabolite of bentazone) was determined to be > 100 mg/L based on nominal concentrations. The NOEC was ≥ 100 mg/L (nominal).

I. MATERIAL AND METHODS

Test item:	Peak B (metabolite of bentazone, Reg. No. 583 1080), batch no. L84-80, purity: 69.9%.
Test species:	Water flea (*Daphnia magna* STRAUS), neonates from in-house culture, >2 < 24 hours old at test initiation.
Test design:	Static system (48 hours), limit test with one test concentration and a control, 4 replicates with 5 daphnids in each; assessment of immobility (and other effects) after 24 and 48 hours.
Endpoints:	EC_{50} and NOEC based on immobility of daphnids.
Test concentrations:	0 (control), 100 mg/L (nominal).
Test conditions:	Glass vessels, test volume 50 mL, dilution water: Elendt "M4" medium; total hardness: 2.41 mmol/L (at test initiation), conductivity: 672 µS/cm (at test initiation), water temperature 21.6°C at the beginning and 20.4 °C at the end of the test; pH value: 7.95 - 8.03; oxygen content: 8.9 mg/L - 9.1 mg/L; photoperiod: 16 hours light : 8 hours dark; light intensity: 226 - 429 lux, no feeding and no aeration.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.

Statistics: Descriptive statistics, Fisher's Exact Binominal Test with Bonferroni Correction for determination of the NOEC. Probit analysis for determination of the EC_{50} value.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in at the beginning and at the end of the test. Measured values for Peak B were 103.2% of nominal concentrations at test initiation and 102.3% at test termination. The analytical data confirmed correct application of the test item. The following biological results are based on nominal concentrations.

Biological results: No immobility occurred at 100 mg Peak B/L and 5% immobility was observed in the control. The results are summarized in Table 8.3/3.

Table 8.3/3 Immobility of *D. magna* resulting from exposure to Peak B (metabolite of bentazone)

Concentration [mg/L] (nominal)	**Control**	**100**
Immobile (24 h) [%]	5	0
Immobile (48 h) [%]	5	0
	Endpoints [mg Peak B/L] (nominal)	
EC_{50} (48 h)	> 100	
NOEC (48 h)	≥ 100	

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of Peak B (metabolite of bentazone) was determined to be > 100 mg/L based on nominal concentrations. The NOEC was ≥ 100 mg/L (nominal).

Reference number:	II A 8.3.1.1/4
Report:	Swierkot A. 2011(a) Reg.No. 5051517 (Metabolite of BAS 351 H, Peak C) Daphnia magna acute immobilization test Institute of Industrial Organic Chemistry; Pszczyna; Poland unpublished BASF DocID 2011/1124106
Guidelines:	OECD 202 (2004); (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to (EC) No 1907/2006 of European Parliament and of Council on the REACH - Part C.2
GLP:	Yes (laboratory certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a static acute toxicity laboratory study, water flea neonates were exposed to Peak C (metabolite of BAS 351 H, M351H024) at nominal concentrations of 5.6, 10.0, 18.0, 32.0, 56.0 and 100.0 mg Peak C/L in 4 replicates per concentration containing 5 daphnids each. Daphnids were observed for immobility 24 hours and 48 hours after start of exposure.

The biological results are based on nominal concentrations of the test item. No immobility occurred at all test item concentrations of Peak C after 24 and 48 hours.

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of Peak C (metabolite of bentazone) was determined to be > 100 mg/L based on nominal concentrations. The NOEC was ≥ 100 mg/L (nominal).

I. MATERIAL AND METHODS

Test item:	Peak C (metabolite of bentazone, Reg. No. 5 051 517), batch no. L84-56, purity: 96% (tolerance ± 1.0%).
Test species:	Water flea (*Daphnia magna* STRAUS), neonates from in-house culture, < 24 hours old at test initiation.
Test design:	Static system (48 hours), 6 test concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility (and other effects) after 24 and 48 hours.
Endpoints:	EC_{50} and NOEC based on immobility of daphnids.
Test concentrations:	Control, 5.6, 10.0, 18.0, 32.0, 56.0 and 100.0 mg Peak C/L (nominal).
Test conditions:	Glass vessels, test volume 50 mL, dilution water: Elendt "M7" medium; temperature: 20.3 °C - 21.0 °C; pH value: 7.74 – 7.84; oxygen content: 8.4 mg/L – 8.6 mg/L; photoperiod: 16 hours light : 8 hours dark; no feeding and no aeration.

Analytics: The content of the test item was determined by high performance liquid chromatography with UV-VIS detection.

Statistics: No statistical analysis was needed in this study.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. At test initiation recoveries of Peak C were in the range of 92.81% to 98.75% of nominal test item concentrations and from 94.91% to 99.64% at test termination. The analytical data confirmed correct application of the test item. The following biological results are based on nominal concentrations.

Biological results: No immobility occurred at all test item concentrations of Peak C after 24 and 48 hours. The results are summarized in Table 8.3/4.

Table 8.3/4 Immobility of *D. magna* resulting from exposure to Peak C (metabolite of bentazone)

Concentration [mg/L] nominal	**Control**	**5.6**	**10.0**	**18.0**	**32.0**	**56.0**	**100.0**
Immobile (24 h) [%]	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Immobile (48 h) [%]	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Endpoints [mg Peak C/L] (nominal)							
EC_{50} (48 h)	> 100						
NOEC (48 h)	≥ 100						

III. CONCLUSION

In a 48-hour static acute toxicity study with *Daphnia magna* the EC_{50} of Peak C (metabolite of bentazone) was determined to be > 100 mg/L based on nominal concentrations. The NOEC was ≥ 100 mg/L (nominal).

8.3.1.2 Acute toxicity (24/48 h) for representative species of aquatic insects

Acute studies with *Daphnia magna* are listed in section 8.3.1.1. Acute studies with other aquatic insects are not required, as direct application to surface water is not foreseen.

8.3.1.3 Acute toxicity for representative species of aquatic crustaceans

Acute studies with *Daphnia magna* are listed in section 8.3.1.1. Acute studies with other aquatic crustaceans are not required because direct application to surface water is not foreseen.

8.3.1.4 Acute toxicity for repr. species of aquatic gastropod molluscs

A study with a representative gastropod mollusc is not required because bentazone is not intended to be used directly in water bodies.

8.3.2 Chronic toxicity to aquatic invertebrates

8.3.2.1 Chronic toxicity in Daphnia magna (21-day)

A 21 day chronic toxicity study on *Daphnia magna* was performed with bentazone (tested with solo-formulation BAS 351 32 H (= Basagran; containing 48% bentazone); Jatzek & Bias, 1989, BASF DocID 1989/10083) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

In the 21-day chronic toxicity study with *Daphnia magna* the NOEC of bentazone (tested as BAS 351 32 H) was 120 mg a.s./L based on nominal concentrations.

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996) and in the EC Review Report (7585/VI/97-final, November 2000) for bentazone.

Additionally, a chronic toxicity study on *Daphnia magna* has been performed with bentazone metabolite N-methyl-bentazone (=M351H009). It is reported here as it contains relevant information and was only recently finalized and not submitted yet in an Annex II dossier.

Reference number:	II A 8.3.2.1/1
Report:	Weltje L., Julier M. 2006(b) Effect of a Bentazone (BAS 351 H) metabolite (BH 351-N-Me, Reg.No. 79520) on survival and reproduction of Daphnia magna STRAUS in a chronic, 21 day, semi-static test BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. unpublished BASF DocID 2005/1013012
Guidelines:	OECD 211; EPA 850.1300
GLP:	Yes (laboratory certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 21-day semi-static toxicity test, effects of N-methyl-bentazone (metabolite of bentazone, M351H009) to water fleas (*Daphnia magna*) were examined. Neonates less than 24 hours old were exposed to nominal concentrations of 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 mg N-methyl-bentazone/L and a control. All treatment groups and the controls consisted of 10 replicates with one parent daphnid in each. After 21 days parent mortality, body length and reproductive performance was assessed.

The biological results were based on nominal concentrations. In the 1.0 mg/L treatment two daphnids died during the course of the study. At 0.5 and 16.0 mg/L each, one parent daphnid died. Parent mortality at 0.5 mg/L and 1.0 mg/L was not considered to be treatment related. The day of first brood ranged from 8 to 9. Reproduction was sufficiently high in all test concentrations and the control except in the two highest test item concentrations. Reproductive performance of parent daphnia was significantly different from the control in the 4.0, 8.0 and 16.0 mg/L treatments, but not in lower concentrations. At 4.0 mg/L the mean number of offspring was significantly higher (Bonferroni t-test) than in the control. This result is not significant according to Williams' test. Statistically significant differences compared to the control in body length of parent daphnia could be detected in the three highest test item treatments (4.0, 8.0 and 16.0 mg/L).

In a 21-day semi-static toxicity study with *Daphnia magna* the NOEC of N-methyl-bentazone (metabolite of BAS 351 H) was determined to be 2.0 mg/L based on nominal concentrations.

I. MATERIAL AND METHODS

Test item:	N-methyl-bentazone (BH 351-N-ME, metabolite of BAS 351 H, Reg. No. 79 520), batch no. 2235-09, purity: 99.8%.
Test species:	Water flea (*Daphnia magna* STRAUS), neonates from in-house culture (originally obtained from Institut National de Recherche Chimique Appliquée, France); ≥ 2 ≤ 24 hours old at test initiation.
Test design:	Semi-static system (21 days), 6 test concentrations plus control, ten replicates per treatment with one parent daphnid in each; assessment of parent mortality, body length and reproduction after 21 days.
Endpoints:	NOEC, parent mortality, reproduction and parent length.
Test concentrations:	Control, 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 mg N-methyl-bentazone/L (nominal).
Test conditions:	Glass vessels, test volume 50 mL, dilution water “M4” (Elendt medium); temperature: 19.9 °C - 21.5 °C; pH 7.86 - 8.60; oxygen content: 8.37 mg/L - 9.25 mg/L; total hardness: 2.40 - 2.49 mmol/L, conductivity: 600 µS/cm - 700 µS/cm; light intensity: < 1500 lux; photoperiod 16 hours light : 8 hours dark; regular feeding with algae (a mixture of *Pseudokirchneriella subcapitata* and *Scenedesmus subspicatus*), no aeration.
Analytics:	The test item concentrations were analyzed using a HPLC-method with UV detection.
Statistics:	Descriptive statistics; Analysis of Variance (ANOVA) followed by Bartlett’s test (α = 0.05), Bonferroni's t-test (α = 0.05) and Williams' test (α = 0.05).

II. RESULTS AND DISCUSSION

Analytical results: Analytical verification of the test item concentrations was conducted in all treatments at day 0, 2, 9, 12, 19 and 21. Mean recoveries of N-methyl-bentazone were in the range of 82.2% - 95.0% of nominal concentrations at test initiation. During the course of the study and at study termination mean recoveries were in the range between 80.5% and 102.6%. The following biological results are based on nominal test item concentrations.

Biological results: In the 1.0 mg/L treatment two daphnids died during the course of the study. At 0.5 and 16.0 mg/L each, one parent daphnid died. Parent mortality at 0.5 mg/L and 1.0 mg/L was not considered to be treatment related. The day of first brood ranged from 8 to 9. Reproduction was sufficiently high in all test concentrations and the control except in the two highest test item concentrations. Reproductive performance of parent daphnia was significantly different from the control in the 4.0, 8.0 and 16.0 mg/L treatments, but not in lower concentrations. At 4.0 mg/L the mean number of offspring was significantly higher (Bonferroni t-test, α = 0.05) than in the control. This result is not significant according to Williams' test (α = 0.05). Statistically significant differences (Williams' test, α = 0.05) compared to the control in body length of parent daphnia could be detected in the three highest test item treatments (4.0, 8.0 and 16.0 mg/L). The results are summarized in Table 8.3/5.

Table 8.3/5 Effects of N-methyl-bentazone (21 d) on *Daphnia magna* reproduction, growth and parent mortality

Concentration [mg/L] nominal	Control	0.5	1.0	2.0	4.0	8.0	16.0
Parent mortality [%]	0	1 [1)]	2 [1)]	0	0	0	1 [1)]
Av. offspring/parent	100.5	112.3	103.4	102.7	124.1 [*]	77.8 [*#]	0 [*#]
Day of first brood	9	8	8	8	8	8	--
Av. body length [mm]	4.40	4.53	4.45	4.35	4.25 [#]	3.93 [#]	1.53 [#]
Endpoints [mg N-methyl-bentazone/L] (nominal)							
$NOEC_{overall}$ (21 d)	2.0						
EC_{50} reproduction (21 d)	> 8.0 < 16.0						
$LC_{50\ parent\ daphnids}$ (21 d)	> 16						

[1)] Replicates with parent mortality were excluded from statistical analysis.
[*] Statistically significant effects compared to the control (ANOVA followed by Bonferroni's t-test).
[#] Statistically significant effects compared to the control (ANOVA followed by Williams' test).
-- could not be determined

III. CONCLUSION

In a 21-day semi-static toxicity study with *Daphnia magna* the NOEC of N-methyl-bentazone (metabolite of BAS 351 H) was determined to be 2.0 mg/L based on nominal concentrations.

8.3.2.2 Chronic toxicity for representative species of aquatic insects

Chronic studies with *Daphnia magna* are listed in chapter 8.3.2.1. Bentazone is not toxic to daphnia (NOEC (21 d) = 120 mg/L) and does not act as insect growth regulator. It does not adsorb to a significant extent to the sediment. Thus, a test on sediment-dwelling organisms is not required.

8.3.2.3 Chronic toxicity for repr. species of aquatic gastropod molluscs

A study with a representative gastropod mollusc is not required because bentazone is not intended to be used directly in water bodies.

8.3.3 Aquatic field testing

Aquatic field testing is not required, since the results of the laboratory studies did not reveal a potential for high risk of bentazone for any aquatic species.

Summary of results

The effects of bentazone and its major metabolites on aquatic invertebrates are summarized in Table 8.3/6.

Table 8.3/6 Summary of results of bentazone and metabolites on aquatic invertebrates

Test species	Test system	Result [mg/L]		Reference
		EC_{50}	NOEC	
Bentazone				
D. magna	Static 48 h	125 #	62.5	Bias, 1986/9009
D. magna	Static 48 h	> 100	≥ 100	Jatzek, 2003/1004524
D. magna *	Semi-static 21 d	--	120	Jatzek & Bias, 1989/10083
Lysimeter leachate +				
D. magna	Static 48 h	no effect	--	Dohmen, 1994/11094
N-methyl-bentazone (=M351H009, Reg. No. 79 520)				
D. magna	Static 48 h	26.5	15.3	Funk, 2004/1003973
D. magna	Semi-static 21 d	--	2.0	Weltje & Julier, 2005/1013012
Peak B (=M351H023, Reg. No. 5 831 080)				
D. magna	Static 48 h	> 100	≥ 100	Dorner, 2011/1163524
Peak C (=M351H024, Reg. No. 5 051 517)				
D. magna	Static 48 h	> 100	≥ 100	Swierkot, 2011/1124106

The endpoint obtained in this study is not supported by analytical measurements. Thus, the result of the new study on *D. magna* by Jatzek (DocID 2003/1004524) is considered valid for acute risk assessment on aquatic invertebrates.
* Study was conducted with the solo formulation BAS 351 32 H (Basagran), containing 48% bentazone.
\+ Study was conducted with leachate waters from BAS 351 32 H treated lysimeters

8.4 Effects on algal growth and growth rate (2 species)

Toxicity studies on green alga have been performed with bentazone and the bentazone metabolite N-methyl-bentazone (= M351H009). They are reported here as they contain relevant information and were not submitted yet in an Annex II dossier.

Reference number:	II A 8.4/1
Report:	Jatzek H.-J. 2003(b) BAS 351 H (Bentazone) - Determination of the inhibitory effect on the cell multiplication of unicellular green algae BASF AG; Ludwigshafen/Rhein; Germany Fed.Rep. unpublished BASF DocID 2003/1012046
Guidelines:	EEC 92/69 A V C 3; OECD 201; EPA 850.5400
GLP:	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In a 72-hour static toxicity laboratory study, the effect of bentazone on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 6.25, 12.5, 25, 50, 100 mg bentazone/L. Assessment of growth was conducted 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations. No morphological effects on algae were observed in the control group and at any of the test item concentrations tested.

In a 72-hour algae test with *Pseudokirchneriella subcapitata* the E_rC_{50} of bentazone was determined to be 33.3 mg/L and the E_bC_{50} was 16.8 mg/L, based on nominal concentrations.

I. MATERIAL AND METHODS

Test item:	Bentazone (BAS 351 H, Reg. no. 51 929), batch no. 14-7887, purity: 98.4%.
Test species:	Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov, specification: SAG 61.81; stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.
Test design:	Static system (72 hours); 5 test concentrations with 3 replicates for each plus a control with 5 replicates; daily assessment of growth.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and biomass development after exposure over 72 hours.
Test concentrations:	Control, 6.25, 12.5, 25, 50, 100 mg bentazone/L (nominal).
Test conditions:	250 mL Erlenmeyer dimple flasks; test volume: 100 mL; nutrient solution (according to OECD 201); pH 6.5 - 8.0 at test initiation and pH 7.8 - 8.2 at test termination; temperature: 23 °C ± 2 °C; initial cell densities: 1 x 10^4 cells/mL; continuous light at about 60 -120 µE/(m²*s), continuous shaking.
Analytics:	Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.
Statistics:	Descriptive statistics, linear regression analysis for determination of EC_x values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for bentazone ranged from 101.2% to 103.8% of nominal at test initiation and from 102.4% to 104.0% of nominal at test termination. As analytical data confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: No morphological effects on algae were observed in the control group and at any of the test item concentrations tested. The results are summarized in **Table 8.4/1**.

Table 8.4/1 Effect of bentazone on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**Control**	**6.25**	**12.5**	**25**	**50**	**100**
Inhibition in 72 h (growth rate) [%] *	--	3.95	13.1	38.0	67.0	96.1
Inhibition in 72 h (biomass) [%] *	--	-0.48	30.6	75.8	94.1	99.3
	Endpoints [mg bentazone/L] (nominal)					
E_rC_{50} (72 h)	33.3					
E_rC_{10} (72 h)	9.89					
E_bC_{50} (72 h)	16.8					
E_bC_{10} (72 h)	7.90					

* Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata* the E_rC_{50} of bentazone was determined to be 33.3 mg/L and the E_bC_{50} was 16.8 mg/L, based on nominal concentrations.

Reference number: II A 8.4/2
Report: Hoffmann F. 2005(b)
Effect of Reg.No. 79520 (BH 351-N-Me) on the growth of the green alga Pseudokirchneriella subcapitata
BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep.
unpublished
BASF DocID 2005/1003989
Guidelines: OECD 201
GLP: Yes
(laboratory certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 72-hour static toxicity laboratory study, the effect of N-methyl-bentazone (metabolite of bentazone, M351H009) on the growth of the green alga *Pseudokirchneriella subcapitata* was investigated. The following nominal concentrations were applied: 3, 6, 12, 24, 48 and 96 mg N-methyl-bentazone/L. Assessment of growth was conducted 24 h, 48 h and 72 h after test initiation.

The biological results are based on nominal concentrations. No morphological effects on algae were observed in the control group and at any of the test item concentrations tested.

In a 72-hour algae test with *Pseudokirchneriella subcapitata* the E_rC_{50} of N-methyl-bentazone was determined to be 37.7 mg/L and the E_yC_{50} was 15.8 mg/L, based on nominal concentrations.

I. MATERIAL AND METHODS

Test item: N-methyl-bentazone (BH 351-N-ME, metabolite of BAS 351 H, Reg. No. 79 520), batch no. 2235-09, purity: 99.8%.

Test species: Unicellular fresh water green alga, *Pseudokirchneriella subcapitata* (Reinsch) Korshikov, specification: SAG 61.81; stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.

Test design: Static system (72 hours); 6 test concentrations with 5 replicates for each plus a control with 10 replicates; daily assessment of growth.

Endpoints: EC_{10} and EC_{50} with respect to growth rate and biomass development after exposure over 72 hours.

Test concentrations: Control, 3, 6, 12, 24, 48 and 96 mg N-methyl-bentazone/L (nominal).

Test conditions: 100 mL Erlenmeyer dimple flasks; test volume: 60 mL; nutrient solution (according to OECD 201); pH 8.1 at test initiation and pH 7.91 - 8.05 at test termination; temperature: 22 °C ± 1 °C; initial cell densities: 1 x 10^4 cells/mL; continuous light at about 8000 lux, continuous shaking.

Analytics: Analytical verification of test item concentrations was conducted using a HPLC-method with UV detection.

Statistics: Descriptive statistics, probit analysis for determination of EC_x values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Mean measured values for N-methyl-bentazone ranged from 87.9% to 94.0% (average 90.7%) of nominal at test initiation and from 91.5% to 99.3% (average 96.2%) of nominal at test termination. As analytical data confirmed correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: No morphological effects on algae were observed in the control group and at any of the test item concentrations tested. The results are summarized in **Table 8.4/2**.

Table 8.4/2 Effect of N-methyl-bentazone (metabolite of bentazone) on the growth of the green alga *Pseudokirchneriella subcapitata*

Concentration [mg/L] (nominal)	**Control**	**3**	**6**	**12**	**24**	**48**	**96**
Inhibition in 72 h (growth rate) [%] *	--	0.0	5.2	13.3	30.4	67.6	76.6
Inhibition in 72 h (biomass) [%] *	--	-0.1	17.1	37.8	67.1	93.3	95.9
	Endpoints [mg N-methyl-bentazone/L] (nominal)						
E_rC_{50} (72 h)	37.7 (95% CL: 36.1 - 39.4)						
E_rC_{10} (72 h)	9.8 (95% CL: 9.2 - 10.4)						
E_yC_{50} (72 h)	15.8 (95% CL: 15.3 - 16.4)						
E_yC_{10} (72 h)	5.2 (95% CL: 4.9 - 5.5)						

* Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 72-hour algae test with *Pseudokirchneriella subcapitata* the E_rC_{50} of N-methyl-bentazone was determined to be 37.7 mg/L and the E_yC_{50} was 15.8 mg/L, based on nominal concentrations.

The following dossier update was made in December 2013 (all changes marked in yellow).

Report:	II A 8.4/3 Hughes J.S., Alexander M.M. 1991(a) The toxicity of Bentazon (BAS 351 H Tech. a.i.) to Anabaena flos-aquae
	BASF DocID 1991/5194
Guidelines:	EPA 123-2
Deviations:	none
GLP:	Yes (laboratory certified by United States Environmental Protection Agency)

Executive Summary

In a 5-day static toxicity laboratory study, the effect of bentazone on biomass development of the blue-green alga *Anabaena flos-aquae* was investigated. The following concentrations were applied: 0 (control), 1.0, 2.0, 4.0, 8.0 and 16.0 mg bentazone/L (nominal, equivalent to mean measured concentrations of 0, 0.778, 1.47, 3.04, 6.28 and 12.10 mg bentazone/L). Assessment of biomass development was conducted on test days 3, 4 and 5.

The biological results are based on mean measured concentrations. After 5 days of exposure no significant effect on algal growth occurred at concentrations up to and including 6.28 mg bentazone/L. In the highest test item concentration 61.4% inhibition compared to the control was observed.

In a 5 day algae test with *Anabaena flos-aquae*, the E_bC_{50} of bentazone was determined to be 10.13 mg/L based on nominal concentrations. The no observed effect concentration (NOEC) was 6.28 mg a.s./L.

I. MATERIAL AND METHODS

Test item:	Bentazone (BAS 351 H, Reg. no. 51 929), batch no. 68-901102; purity: 53.0%
Test species:	Unicellular fresh water blue-green alga, *Anabaena flos-aquae*; in-house culture; stock originally obtained from "The American Type Culture Collection", Rockville, MD, USA.
Test design:	Static system (5 days); 5 test concentrations with 3 replicates for each test item concentration and the control; assessment of growth on days 3, 4 and 5.
Endpoints:	EC_{25}, EC_{50} and NOEC with respect to biomass development after exposure over 5 days.
Test concentrations:	Control, 1.0, 2.0, 4.0, 8.0 and 16.0 mg bentazone/L (nominal), corresponding to mean measured concentrations of < 0.10 (control), 0.778, 1.47, 3.04, 6.28 and 12.10 mg bentazone/L.
Test conditions:	500 mL Erlenmeyer flasks plugged with gas permeable foam plugs; test volume: 100 mL; AAP nutrient medium; pH 7.20 - 7.42 at test initiation and pH 7.77 - 7.93 at test termination; temperature: 24 °C ± 2 °C; initial cell densities: 3 x 10^3 cells/mL; continuous light at 2153 ± 323 lux, manual shaking once each working day.
Analytics:	Analytical verification of test item concentrations was conducted using a gas chromatographic procedure with nitrogen-phosphororus detection.
Statistics:	Descriptive statistics, nonlinear regression analysis for determination of EC_x values, Dunnett's test for determination of NOEC value.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Measured concentrations of bentazone ranged from 80% to 88% of nominal at test initiation and from 67% to 74% of nominal at test termination. The test material may have undergone degradation during the study, may have adsorbed to the glassware or the algae, or may have been taken up by the algae. As the concentration of bentazone was not maintained during the test, the following biological results are based on mean measured concentrations.

Biological results: After 5 days of exposure no significant effect on algal growth occurred at concentrations up to and including 6.28 mg bentazone/L. In the highest test item concentration 61.4% inhibition compared to the control was observed. The effects on algal biomass are summarized in Table 8.4/3.

Table 8.4/3 Effect of bentazone on biomass development of the blue-green alga *Anabaena flos-aquae*

Concentration [mg a.s./L] (nominal)	**1.0**	**2.0**	**4.0**	**8.0**	**16.0**
Concentration [mg a.s./L] (mean measured)	**0.778**	**1.47**	**3.04**	**6.28**	**12.10**
Inhibition in cell density compared with control after 5 days [%] *	-4.7	-4.9	1.7	17.4	61.4 #
	Endpoints [mg a.s./L] (mean measured)				
E_bC_{50} (5 d)	10.13 (95% confidence limits: 9.22 - 11.31)				
E_bC_{25} (5 d)	6.90 (95% confidence limits: 5.79 - 8.21)				
NOEC (5 d)	6.28				

* Negative values indicate stimulated growth compared to the control.
# Statistically significant differences compared to control (Dunnett's test, $\alpha = 0.05$).

III. CONCLUSION

In a (5 d) algae test with *Anabaena flos-aquae*, the E_bC_{50} of bentazone was determined to be 10.13 mg/L based on nominal concentrations. The no observed effect concentration (NOEC) was 6.28 mg a.s./L.

Report:	II A 8.4/4 Hughes J.S., Alexander M.M. 1991(b) The toxicity of Bentazon (BAS 351 H Tech. a.i.) to Navicula pelliculosa
	BASF DocID 1991/5198
Guidelines:	EPA 123-2
Deviations:	none
GLP:	Yes (laboratory certified by United States Environmental Protection Agency)

Executive Summary

In a 5-day static toxicity laboratory study, the effect of bentazone on the growth of the unicellular fresh water diatom *Navicula pelliculosa* was investigated. The following nominal concentrations were applied: 0 (control), 0.5, 1, 2, 4, 8 and 16 mg bentazone/L (equivalent to mean measured concentrations of 0, 0.52, 0.96, 1.72, 3.60, 7.30 and 14.73 mg a.s./L). Assessment of growth was conducted on days 3, 4 and 5 after test initiation.

The biological results are based on mean measured concentrations. No statistically significant differences compared to the control were observed at any test item concentration.

In a 5-day algae test with *Navicula pelliculosa,* the E_bC_{50} (5 d) for bentazone was determined to be > 14.73 mg a.s./L and the NOEC was determined to be 14.73 mg a.s./L, based on mean measured concentrations.

I. MATERIAL AND METHODS

Test item:	Bentazone (BAS 351 H, Reg. no. 51 929), batch no. 68-901102; purity: 53.0%
Test species:	Unicellular fresh water diatom, *Navicula pelliculosa*, UTEX #667; ; in-house culture, stock originally obtained from the "The Culture Collection of Algae" University of Texas at Austin, USA.
Test design:	Static system; test duration 5 days; 6 test concentrations, each with 4 replicates per treatment plus a control with 4 replicates; assessment of growth on days 3, 4 and 5.
Endpoints:	EC_{25}, EC_{50} and NOEC with respect to biomass after exposure over 5 days.
Test concentrations:	Control, 0.5, 1, 2, 4, 8 and 16 mg bentazone/L (nominal); equivalent to mean measured concentrations of 0, 0.52, 0.96, 1.72, 3.60, 7.30 and 14.73 mg a.s./L.
Test conditions:	250 mL glass Erlenmeyer dimple flasks; test volume 50 mL; synthetic AAP/Si medium; pH 7.43 - 7.53 at test initiation and pH 7.62 - 7.84 at test termination; temperature: 24.0 ± 2 °C; initial cell densities 3 x 10^3 cells/mL; continuous light at 4306 ± 646 lux; constant shaking.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.
Statistics:	Descriptive statistics; ANOVA and Dunnett's test for determination of the NOEC value (α = 0.05).

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each concentration at the beginning and at the end of the test. The analyzed contents of bentazone ranged from 88% to 105% of nominal at test initiation and from 84% to 103% of nominal at test termination. The following biological results are based on mean measured concentrations.

Biological results: No statistically significant differences compared to the control were observed at any test item concentration (ANOVA and Dunnett's test, α = 0.05). The effects on algal growth are summarized in Table 8.4/4.

Table 8.4/4 Effect of bentazone on the growth of the unicellular fresh water diatom *Navicula pelliculosa*

Concentration [mg a.s./L] (nominal)	**0.5**	**1.0**	**2.0**	**4.0**	**8.0**	**16.0**
Concentration [mg a.s./L] (mean measured)	**0.52**	**0.96**	**1.72**	**3.60**	**7.30**	**14.73**
Inhibition in cell density compared with control after 5 days [%]*	30.5	51.1	35.5	16.5	-55.1	-40.7
Endpoints [mg bentazone/L] (nominal)						
E_bC_{50} (5 d)	> 14.73 #					
E_bC_{25} (5 d)	> 14.73 #					
NOEC (5 d)	14.73					

* Negative values indicate stimulated growth compared to the control.
# EC_{25} and EC_{50} values were not determined, since no dose-response pattern was observed.

III. CONCLUSION

In a 5-day algae test with *Navicula pelliculosa*, the E_bC_{50} (5 d) for bentazone was determined to be > 14.73 mg a.s./L and the NOEC was determined to be 14.73 mg a.s./L, based on mean measured concentrations.

Report:	II A 8.4/5 Hughes J.S., Alexander M.M. 1991(c) The toxicity of Bentazon (BAS 351 H Tech. a.i.) to Skeletonema costatum (Tier 2 growth and reproduction of aquatic plants) BASF DocID 1991/5199
Guidelines:	EPA 123-2
Deviations:	none
GLP:	Yes (laboratory certified by United States Environmental Protection Agency)

Executive Summary

In a 5-day static toxicity laboratory study, the effect of bentazone on biomass development of the marine diatom *Skeletonema costatum* was investigated. The following concentrations were applied: 0 (control), 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 mg bentazone/L (nominal, equivalent to mean measured concentrations of 0, 0.54, 1.00, 1.83, 3.72, 7.14 and 14.35 mg bentazone/L). Assessment of biomass was conducted on test days 3, 4 and 5.

The biological results are based on mean measured concentrations. After 5 days of exposure no significant effect on algal growth occurred at concentrations up to and including 3.72 mg bentazone/L. At the two highest test item concentrations of 7.14 mg and 14.35 mg 30.1% and 70.5% inhibition compared to the control were observed, respectively.

In a 5-day algae test with *Skeletonema costatum*, the E_bC_{50} of bentazone was determined to be 10.09 mg/L based on mean measured concentrations.

I. MATERIAL AND METHODS

Test item:	Bentazone (BAS 351 H, Reg. no. 51 929), batch no. 68-901102; purity: 53.0%
Test species:	Marine diatom, *Skeletonema costatum*, in-house culture; stock originally obtained from the "EPA Environmental Research Laboratory", Gulf Breeze, Florida, USA.
Test design:	Static system (120 hours); 6 test concentrations with 3 replicates for each test item concentration and the control; assessment of biomass on days 3, 4 and 5.
Endpoints:	EC_{25}, EC_{50} and NOEC with respect to biomass after exposure over 5 days.
Test concentrations:	Control, 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 mg bentazone/L (nominal), corresponding to mean measured concentrations of < 0.10 (control), 0.54, 1.00, 1.83, 3.72, 7.14 and 14.35 mg bentazone/L.
Test conditions:	250 mL Erlenmeyer flasks plugged with gas permeable foam plugs; test volume: 50 mL; synthetic MAA medium; pH 8.03 - 8.06 at test initiation and pH 7.29 - 7.48 at test termination; temperature: 20 °C ± 2 °C; initial cell densities: 1 x 10^4 cells/mL; continuous light at 4306 ± 646 lux, manual shaking once each working day.
Analytics:	Analytical verification of test item concentrations was conducted using an HPLC-method with UV detection.
Statistics:	Descriptive statistics, nonlinear regression analysis for determination of EC_x values, Dunnett's test for determination of NOEC value.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Measured concentrations of bentazone ranged from 77% to 102% of nominal at test initiation and from 88% to 115% of nominal at test termination. As the concentrations of bentazone during the test were not maintained within ± 20% variation, the following biological results are based on mean measured concentrations.

Biological results: After 5 days of exposure no significant effect on biomass development occurred at concentrations up to and including 3.72 mg bentazone/L. At the two highest test item concentrations of 7.14 mg and 14.35 mg 30.1% and 70.5% inhibition compared to the control were observed, respectively. The effects on algal biomass are summarized in Table 8.4/5.

Table 8.4/5 Effect of bentazone on the biomass of the marine diatom *Skeletonema costatum*

Concentration [mg a.s./L] (nominal)	**0.5**	**1.0**	**2.0**	**4.0**	**8.0**	**16.0**
Concentration [mg a.s./L] (mean measured)	**0.54**	**1.00**	**1.83**	**3.72**	**7.14**	**14.35**
Inhibition in cell density compared with control after 120 hours [%]	**13.9**	14.7	15.8	13.4	30.1 *	70.5 *
	Endpoints [mg a.s./L] (mean measured)					
E_bC_{50} (120 h)	10.09 (95% confidence limits: 7.68 - 13.26)					
E_bC_{25} (120 h)	6.31 (95% confidence limits: 3.94 - 10.11)					
NOEC (120 h)	3.72					

* Statistically significant differences compared to control (Dunnett's test, α = 0.05).

III. CONCLUSION

In a 5-day algae test with *Skeletonema costatum*, the E_bC_{50} of bentazone was determined to be 10.09 mg/L based on mean measured concentrations.

Furthermore, a toxicity study on the green alga *Ankistrodesmus bibraianus* was performed with bentazone (Dohmen, 1990, BASF DocID 1990/0167) and was assessed in the EU review of bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

In a 72-hour algae test with *Ankistrodesmus bibraianus* the EC_{50} of bentazone was graphically determined to be 62 mg/L.

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996). **However, since the test concentrations in the study were not analytically verified, the study result is not considered further in the risk assessment.**

A further toxicity study on the green alga *Ankistrodesmus bibraianus* was performed with the DEA-salt of bentazone (Dohmen, 1990, BASF DocID 1990/0166) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

In a 72-hour algae test with *Ankistrodesmus bibraianus* the EC_{50} of bentazone was graphically determined to be 71 mg/L (corresponding to 102 mg bentazone DEA-salt/L).

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996). **However, since the test concentrations in the study were not analytically verified, the study result is not considered further in the risk assessment.**

A toxicity study on the green alga *Pseudokirchneriella subcapitata* was performed with bentazone (tested with the lysimeter leachate of the formulation BAS 351 32 H; Dohmen, 1994, BASF DocID 1994/11093) and was assessed in the EU review of bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

The observed differences in algal performance in the three different leachate waters are within the normal range of variability which can be expected when leachates from different lysimeters are tested. There is no indication that potential bentazone derived residues in leachates are of any ecotoxicological concern to green algae.

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996).

Summary of results

The effects of bentazone and its metabolite N-methyl-bentazone (= M351H009) on algae are summarized in **Table 8.4/6**.

Table 8.4/6 **Summary of results of bentazone and its metabolite N-methyl-bentazone on aquatic invertebrates**

Test species	Test system	Result [mg/L]		Reference
		EC_{50}	NOEC	
Bentazone				
P. subcapitata	Static 72 h	33.3 [1] 16.8 [2]	9.89 [3] 7.90 [4]	Jatzek, 2003/1012046
A. bibraianus	Static 72 h	62 [#]	1.5 [4]	Dohmen, 1990/0167
A. bibraianus *	Static 72 h	71 [#]	5.0 [4]	Dohmen, 1990/0166
A. flos aquae	Static 120 h	10.13 [2]	6.28	Hughes & Alexander 1991/5194
N. pelliculosa	Static 120 h	> 14.73 [2]	14.73	Hughes & Alexander 1991/5198
S. costatum	Static 120 h	10.09 [2]	3.72	Hughes & Alexander, 1991/5199
Lysimeter leachate [+]				
P. subcapitata	Static 72 h	no significant effects	--	Dohmen, 1994/11093
N-methyl-bentazone (= M351H009,Reg. No. 79 520)				
P. subcapitata	Static 72 h	37.7 [1] 15.8 [2]	9.8 [3] 5.2 [4]	Hoffmann, 2005/1003989

[#] graphical determination
* Study was conducted with the DEA-salt of bentazone; the endpoint presented was calculated on the basis of free bentazone (corresponding to 102 mg bentazone DEA-salt/L).
[+] Study was conducted with leachate waters from BAS 351 32 H treated lysimeters
[1] E_rC_{50} [2] E_bC_{50} / E_yC_{50} [3] E_rC_{10} [4] E_bC_{10} / E_yC_{10}

8.5 Effects on sediment dwelling organisms

Bentazone is not toxic to daphnia (NOEC (21 d) = 120 mg/L; see chapter 8.3.2). It does not adsorb to a significant extent to the sediment. Thus a test on sediment-dwelling organisms is not warranted.

8.5.1 Acute test

Not required (see above).

8.5.2 Chronic test

Not required (see above).

8.6 Effects on aquatic plants

Toxicity studies on the aquatic plant *Lemna gibba* have been performed with bentazone, the bentazone Na-salt and the bentazone metabolites N-methyl-bentazone (= M351H009), Peak B (= M351H023) and Peak C (= M351H024). They are reported here as they contain relevant information and were only recently finalized and not submitted yet in an Annex II dossier.

Reference number:	II A 8.6/1
Report:	Hoffmann F. 2011(b) Effect of Bentazone (Reg.No. 51929) on the growth of Lemna gibba BASF SE; Limburgerhof; Germany Fed.Rep. unpublished BASF DocID 2011/1102365
Guidelines:	OECD 221; EPA 850.4400 (draft 1996); ASTM E 1415-91
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 7-day static toxicity laboratory study, the effect of bentazone on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0 (control), 0.410, 1.23, 3.70, 11.1, 33.3 and 100 mg bentazone/L. Assessment of growth and other effects was conducted 3, 5 and 7 days after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration based upon mean growth rate data and final yield for the parameters frond number and dry weight.

The biological results are based on nominal concentrations. The duckweed population in the control vessels showed sufficient growth, increasing from 11 fronds per vessel to an average of 134.0 fronds per vessel, corresponding to an 11.2 x multiplication. The dry weight increased from 1.7 mg to an average of 15.0 mg per vessel in the control at test termination. No morphological effects were observed in the control group and at tested concentrations of up to and including 3.70 mg bentazone/L. At the concentration of 11.1 mg/L, 33.3 mg/L and 100 mg/L about two third of the fronds appeared smaller and one frond was necrotic from day 5 on. At the highest test concentration roots appeared very small at test termination.

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} of bentazone was 25.0 mg a.s./L based on frond number and 12.0 mg a.s./L based on dry weight. The E_yC_{50} was 9.2 mg a.s./L based on frond number and 7.3 mg a.s./L based on dry weight (nominal).

I. MATERIAL AND METHODS

Test item:	Bentazone (BAS 351 H, Reg. No. 51 929), batch no. COD-001416, purity: 100.0% (tolerance ± 1.0%).
Test species:	Duckweed (*Lemna gibba* G3), inocula 7 - 10 days old cultures; cultures maintained in-house; stock obtained from "ÖkoTox Moser & Pickl GbR", Stuttgart, Germany.
Test design:	Static system (7 days); 7 treatment groups (6 test item concentrations, control) with 3 replicates for the test item treatments and 6 replicates for the control; 2 plants with 4 fronds and 1 plant with 3 fronds, total number of fronds at test initiation: 11 per replicate; assessment of growth and other effects on days 3, 5 and 7.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 7 days.
Test concentrations:	Control, 0.410, 1.23, 3.70, 11.1, 33.3 and 100 mg bentazone/L (nominal).
Test conditions:	400 mL glass beakers, test volume 160 mL, 20x-AAP nutrient medium, pH 7.48 - 7.53 at test initiation and pH 8.24 - 8.45 at test termination; mean water temperature: 24.2 °C, continuous light, average light intensity: about 8.3 klux.
Analytics:	Analytical verification of the test item was conducted using a HPLC-method with UV detection.
Statistics:	Descriptive statistics, probit analysis for determination of EC_x values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Measured values for bentazone ranged from 99.0% to 105.1% of the nominal concentration at test initiation and from 78.4% to 104.8% the nominal concentration at test termination. As initially measured concentrations confirmed correct application of the test substance, the following biological results are based on nominal concentrations

Biological results: The duckweed population in the control vessels showed sufficient growth, increasing from 11 fronds per vessel to an average of 134.0 fronds per vessel, corresponding to an 11.2 x multiplication. The dry weight increased from 1.7 mg to an average of 15.0 mg per vessel in the control at test termination. No morphological effects were observed in the control group and at tested concentrations of up to and including 3.70 mg bentazone/L. At the concentration of 11.1 mg/L, 33.3 mg/L and 100 mg/L about two third of the fronds appeared smaller and one frond was necrotic from day 5 on. At the highest test concentration roots appeared very small at test termination. Effects on growth rate and yield are summarized in Table 8.6/1.

Table 8.6/1 Effect of bentazone on the growth of duckweed *Lemna gibba*

Concentration [mg/L](nominal)	**0.410**	**1.23**	**3.70**	**11.1**	**33.3**	**100**
Inhibition after 7 d [%] * (growth rate based on frond no.)	-1.2	-1.0	1.6	36.1	62.7	73.0
Inhibition after 7 d [%] * (growth rate based on dry weight)	0.1	0.3	5.3	52.8	80.7	94.9
Inhibition after 7 d [%] * (yield based on frond no.)	-3.3	-2.7	4.3	64.8	86.2	91.3
Inhibition after 7 d [%] * (yield based on dry weight)	0.3	0.8	12.3	76.9	93.2	98.5

	Endpoints [mg bentazone/L] (nominal)
E_rC_{50} (7 d) based on frond number	25.0 (95% CL: 20.3 - 31.1)
E_rC_{10} (7 d) based on frond number	3.3 (95% CL: 1.9 - 4.8)
E_yC_{50} (7 d) based on frond number	9.2 (95% CL: 7.9 - 10.6)
E_yC_{10} (7 d) based on frond number	3.8 (95% CL: 2.4 - 4.8)
E_rC_{50} (7 d) based on dry weight	12.0 (95% CL: 10.6 - 13.7)
E_rC_{10} (7 d) based on dry weight	3.4 (95% CL: 2.5 - 4.3)
E_yC_{50} (7 d) based on dry weight	7.3 (95% CL: 6.8 - 7.8)
E_yC_{10} (7 d) based on dry weight	3.4 (95% CL: 2.9 - 3.8)

95% CL = 95% confidence limits
* Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} of bentazone was 25.0 mg a.s./L based on frond number and 12.0 mg a.s./L based on dry weight (nominal). The E_yC_{50} was 9.2 mg a.s./L based on frond number and 7.3 mg a.s./L based on dry weight (nominal).

Reference number:	II A 8.6/2
Report:	Hoffmann F. 2011(a) Effect of Bentazone-Na (Reg.No. 88691) on the growth of Lemna gibba BASF SE; Limburgerhof; Germany Fed.Rep. unpublished BASF DocID 2011/1102366
Guidelines:	OECD 221; EPA 850.4400 (draft 1996); ASTM E 1415-91
GLP:	Yes (laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 7-day static toxicity laboratory study, the effect of bentazone-Na on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0 (control), 0.410, 1.23, 3.70, 11.1, 33.3 and 100 mg bentazone-Na/L. Assessment of growth and other effects was conducted 3, 5 and 7 days after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration based upon mean growth rate data and final yield for the parameters frond number and dry weight.

The biological results are based on nominal concentrations. The duckweed population in the control vessels showed sufficient growth, increasing from 11 fronds per vessel to an average of 123.5 fronds per vessel, corresponding to an 11.2 x multiplication. The dry weight increased from 1.8 mg to an average of 16.2 mg per vessel in the control at test termination. No morphological effects on algae were observed in the control group and at tested concentrations of up to and including 1.23 mg/L. At the concentration of 3.70 mg/L and 11.1 mg/L about one third of the fronds appeared smaller from day 5 on. At the two highest tested concentrations of 33.3 mg/L and 100 mg/L two third of the fronds were smaller from day 5 on and the roots appeared were smaller at test termination.

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} of bentazone-Na was 22.2 mg/L based on frond number and 18.1 mg/L based on dry weight (nominal). The E_yC_{50} was 9.7 mg bentazone-Na/L based on frond number and 8.7 mg/L based on dry weight number (nominal).

I. MATERIAL AND METHODS

Test item:	Bentazone-Na (Reg. No. 88 691), batch no. COD-001417, purity: 91.9% (tolerance ± 1.0%).
Test species:	Duckweed (*Lemna gibba* G3), inocula 7 - 10 days old cultures; cultures maintained in-house; stock obtained from "ÖkoTox Moser & Pickl GbR", Stuttgart, Germany.
Test design:	Static system (7 days); 6 treatment groups (5 test item concentrations, control) with 3 replicates for the test item treatments and 6 replicates for the control; 2 plants with 4 fronds and 1 plant with 3 fronds, total number of fronds at test initiation: 11 fronds per replicate; assessment of growth and other effects on days 3, 5 and 7.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 7 days.
Test concentrations:	Control, 0.410, 1.23, 3.70, 11.1, 33.3 and 100 mg bentazone-Na/L (nominal).
Test conditions:	400 mL glass beakers, test volume 160 mL, 20x-AAP nutrient medium, pH 7.48 - 7.52 at test initiation and pH 8.24 - 8.47 at test termination; mean water temperature: 24.2 °C, continuous light, average light intensity: about 8.3 klux.
Analytics:	Analytical verification of the test item was conducted using a HPLC-method with UV detection.
Statistics:	Descriptive statistics, probit analysis for determination of EC_x values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Measured values for bentazone-Na ranged from 101.6% to 106.5% of nominal at test initiation and from 74.8% to 110.3% of nominal at test termination. As initially measured concentrations confirmed correct application of the test substance, the following biological results are based on nominal concentrations.

Biological results: The duckweed population in the control vessels showed sufficient growth, increasing from 11 fronds per vessel to an average of 123.5 fronds per vessel, corresponding to an 11.2 x multiplication. The dry weight increased from 1.8 mg to an average of 16.2 mg per vessel in the control at test termination. No morphological effects on algae were observed in the control group at nominal test concentrations of up to and including 1.23 mg/L. At the concentration of 3.70 mg/L and 11.1 mg/L about one third of the fronds appeared smaller from day 5 on. At the two highest tested concentrations of 33.3 mg/L and 100 mg/L two third of the fronds were smaller from day 5 on and the roots appeared were smaller at test termination. Effects on growth rate and yield are summarized in Table 8.6/2.

Table 8.6/2 Effect of bentazone-Na on the growth of duckweed *Lemna gibba*

Concentration [mg/L](nominal)	**0.410**	**1.23**	**3.70**	**11.1**	**33.3**	**100**
Inhibition after 7 d [%] * (growth rate based on frond no.)	-3.6	-0.3	3.1	33.3	65.6	80.5
Inhibition after 7 d [%] * (growth rate based on dry weight)	0.0	0.7	5.5	41.1	63.3	94.5
Inhibition after 7 d [%] * (yield based on frond no.)	-9.9	-0.7	7.9	60.6	87.3	94.1
Inhibition after 7 d [%] * (yield based on dry weight)	0.0	1.8	12.9	66.7	84.5	98.4
	Endpoints [mg bentazone-Na/L] (nominal)					
E_rC_{50} (7 d) based on frond number	22.2 (95% CL: 19.2 - 25.6)					
E_rC_{10} (7 d) based on frond number	3.9 (95% CL: 2.7 - 5.1)					
E_yC_{50} (7 d) based on frond number	9.7 (95% CL: 8.7 - 10.9)					
E_yC_{10} (7 d) based on frond number	3.4 (95% CL: 2.5 - 4.1)					
E_rC_{50} (7 d) based on dry weight	18.1 (95% CL: 15.8 - 20.7)					
E_rC_{10} (7 d) based on dry weight	3.6 (95% CL: 2.6 - 4.7)					
E_yC_{50} (7 d) based on dry weight	8.7 (95% CL: 7.6 - 9.9)					
E_yC_{10} (7 d) based on dry weight	2.7 (95% CL: 1.9 - 3.4)					

95% CL = 95% confidence limits
* Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} of bentazone-Na was 22.2 mg/L based on frond number and 18.1 mg/L based on dry weight (nominal). The E_yC_{50} was 9.7 mg bentazone-Na/L based on frond number and 8.7 mg/L based on dry weight number (nominal).

Reference number:	II A 8.6/3
Report:	Junker M. 2004(b) Effect of Reg.No. 79520 (metabolite of BAS 351 H, Bentazone) on the growth of Lemna gibba BASF AG Agrarzentrum Limburgerhof; Limburgerhof; Germany Fed.Rep. unpublished BASF DocID 2004/1003981
Guidelines:	OECD draft guideline (Oct. 2000) Lemna sp. growth inhibition test; EPA 850.4400; ASTM E 1415-91
GLP:	Yes (laboratory certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 7-day static toxicity laboratory study, the effect of N-methyl-bentazone (metabolite of bentazone, M351H009) on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0 (control), 3.13, 6.25, 12.5, 25, 50 and 100 mg N-methyl-bentazone/L. Assessment of growth and other effects was conducted 3, 5 and 7 days after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration based upon mean growth rate data and mean biomass data for the parameters frond number and dry weight.

The biological results are based on mean measured concentrations. The duckweed population in the control vessels grew well, increasing from 11 fronds per vessel to an average of 175.83 fronds per vessel, corresponding to a 16 x multiplication. The dry weight increased from 1.10 mg to an average of 21.76 mg per vessel in the control at test termination. No morphological effects were observed in the control group and at tested mean measured concentrations of up to and including 17.54 mg N-methyl-bentazone/L. At the concentration of 39.19 mg/L the new fronds remained smaller at the end of the study. At the highest test concentration the fronds were chlorotic on day 7.

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} for N-methyl-bentazone (metabolite of bentazone) was 35.84 mg/L based on frond number and 42.99 mg/L based on dry weight (mean measured). The E_yC_{50} was 21.32 mg/L based on frond number and 25.08 mg/L based on dry weight were (mean measured).

I. MATERIAL AND METHODS

Test item:	N-methyl-bentazone (Reg. No. 79520; metabolite of bentazone), batch no. 2235-09, purity: 99.8%.
Test species:	Duckweed (*Lemna gibba* G3), inocula 7 - 10 days old cultures; cultures maintained in-house; stock obtained from "ÖkoTox Moser & Pickl GbR", Stuttgart, Germany.
Test design:	Static system (7 days); 7 treatment groups (6 test item concentrations, control) with 3 replicates for the test item treatments and 6 replicates for the control; 2 plants with 4 fronds and 1 plant with 3 fronds, total number of fronds at test initiation: 11 per replicate; assessment of growth and other effects on days 3, 5 and 7.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and biomass after exposure over 7 days.
Test concentrations:	Control, 3.13, 6.25, 12.5, 25, 50 and 100 mg N-methyl-bentazone/L (nominal); corresponding to mean measured concentrations of 1.82, 3.83, 8.20, 17.54, 39.19 and 90.98 mg N-methyl-bentazone/L.
Test conditions:	400 mL glass beakers, test volume 160 mL, 20x-AAP nutrient medium, pH 7.52 - 7.57 at test initiation and pH 7.63 - 7.87 at test termination; water temperature: 25 - 26 °C, continuous light, light intensity: between 8.11 - 8.98 klux.
Analytics:	Analytical verification of the test item was conducted using a HPLC-method with UV detection.
Statistics:	Descriptive statistics, analysis of variance, Bonferroni test, probit analysis for determination of EC_x values.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Measured values for Reg. No. 79520 ranged from 84.7% to 98.3% of the nominal concentration at test initiation and from 30.9% to 83.7% of the nominal concentration at test termination. The following biological results are based on mean measured concentrations.

Biological results: The duckweed population in the control vessels grew well, increasing from 11 fronds per vessel to an average of 175.83 fronds per vessel, corresponding to a 16 x multiplication. The dry weight increased from 1.10 mg to an average of 21.76 mg per vessel in the control at test termination. No morphological effects were observed in the control group and at tested mean measured concentrations of up to and including 17.54 mg N-methyl-bentazone/L. At the concentration of 39.19 mg/L the new fronds remained smaller at the end of the study. At the highest test concentration the fronds were chlorotic on day 7. Effects on growth rate and yield are summarized in Table 8.6/3.

Table 8.6/3 Effect of N-methyl-bentazone (metabolite of bentazone) on the growth of duckweed *Lemna* gibba

Concentration [mg/L](nominal)	**3.13**	**6.25**	**12.5**	**25**	**50**	**100**
Concentration [mg/L](mean measured)	**1.82**	**3.83**	**8.20**	**17.54**	**39.19**	**90.98**
Inhibition after 7 d [%] * (growth rate based on frond no.)	-0.2	-0.1	2.9	16.8	52.7	90.7
Inhibition after 7 d [%] * (growth rate based on dry weight)	-1.6	-1.4	0.0	14.5	41.2	85.9
Inhibition after 7 d [%] * (biomass based on frond no.)	-0.5	-0.3	8.2	39.7	81.8	98.0
Inhibition after 7 d [%] * (biomass based on dry weight)	-4.9	-4.4	0.0	37.0	74.3	97.1
	Endpoints [mg N-methyl-bentazone/L] (mean measured)					
E_rC_{50} (7 d) based on frond number	35.84 (95% CL: 34.54 - 37.18)					
E_rC_{10} (7 d) based on frond number	13.93 (95% CL: 13.19 - 14.71)					
E_bC_{50} (7 d) based on frond number	21.32 (95% CL: 20.61 - 22.05)					
E_bC_{10} (7 d) based on frond number	9.0 (95% CL: 8.55 - 9.47)					
E_rC_{50} (7 d) based on dry weight	42.99 (95% CL: 41.43 - 44.62)					
E_rC_{10} (7 d) based on dry weight	17.1 (95% CL: 16.18 - 18.06)					
E_bC_{50} (7 d) based on dry weight	25.08 (95% CL: 24.28 - 25.91)					
E_bC_{10} (7 d) based on dry weight	11.31 (95% CL: 10.75 - 11.89)					

95% CL = 95% confidence limits
* Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} for N-methyl-bentazone (metabolite of bentazone) was 35.84 mg/L based on frond number and 42.99 mg/L based on dry weight (mean measured). The E_yC_{50} was 21.32 mg/L based on frond number and 25.08 mg/L based on dry weight were (mean measured).

Reference number: II A 8.6/4
Report: Hoffmann F. 2011(c)
Effect of Reg.No. 5831080 (metabolite of Bentazone, BAS 351 H) on the growth of Lemna gibba
BASF SE; Limburgerhof; Germany Fed.Rep.
unpublished
BASF DocID 2011/1165915
Guidelines: OECD 221; EPA 850.4400 (draft 1996); ASTM E 1415-91
GLP: Yes
(laboratory certified by Landesamt fuer Umwelt, Wasserwirtschaft und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

In a 7-day static toxicity laboratory study, the effect of Peak B (metabolite of bentazone, M351H023) on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0 (control), 0.410, 1.23, 3.70, 11.1, 33.3 and 100 mg Peak B/L. Assessment of growth and other effects was conducted 3, 5 and 7 days after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration based upon mean growth rate data and final yield for the parameters frond number and dry weight.

The biological results are based on nominal concentrations. The duckweed population in the control vessels showed exponential growth, increasing from 11 fronds per vessel to an average of 183 fronds per vessel, corresponding to a 16.6 x multiplication. The dry weight increased from 1.8 mg to an average of 25.1 mg per vessel in the control at test termination. No morphological effects were observed in the control group and any test item concentration.

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} and E_yC_{50} of Peak B (metabolite of bentazone) were determined to be > 100 mg /L based on both, frond number and dry weight (nominal).

I. MATERIAL AND METHODS

Test item:	Peak B (metabolite of bentazone, Reg. No. 583 1080), batch no. L84-80, purity: 69.9%.
Test species:	Duckweed (*Lemna gibba* G3), inocula 7 - 10 days old cultures; cultures maintained in-house; stock obtained from "ÖkoTox Moser & Pickl GbR", Stuttgart, Germany.
Test design:	Static system (7 days); 7 treatment groups (6 test item concentrations, control) with 3 replicates for the test item treatments and 6 replicates for the control; 2 plants with 4 fronds and 1 plant with 3 fronds, total number of fronds at test initiation: 11 per replicate; assessment of growth and other effects on days 3, 5 and 7.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 7 days.
Test concentrations:	Control, 0.41, 1.23, 3.70, 11.1, 33.3 and 100 mg Peak B/L (nominal).
Test conditions:	400 mL glass beakers, test volume 160 mL, 20x-AAP nutrient medium, pH 7.50 - 7.53 at test initiation and pH 8.42 - 8.52 at test termination; mean water temperature: 24.1 °C, continuous light, average light intensity: about 8.2 klux.
Analytics:	Analytical verification of the test item was conducted using a HPLC-method with UV detection.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Measured values for Peak B ranged from 99.7% to 104.4% of the nominal concentration at test initiation and from 94.9% to 112.4% of the nominal concentration at test termination. As analytical data confirmed generally the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: The duckweed population in the control vessels showed exponential growth, increasing from 11 fronds per vessel to an average of 183 fronds per vessel, corresponding to a 16.6 x multiplication. The dry weight increased from 1.8 mg to an average of 25.1 mg per vessel in the control at test termination. No morphological effects were observed in the control group and any test item concentration. Effects on growth rate and yield are summarized in Table 8.6/4.

Table 8.6/4 Effect of Peak B (metabolite of bentazone) on the growth of duckweed *Lemna gibba*

Concentration [mg/L](nominal)	**0.410**	**1.23**	**3.70**	**11.1**	**33.3**	**100**
Inhibition after 7 d [%] * (growth rate based on frond no.)	-0.3	-0.4	0.0	-0.5	-0.6	0.9
Inhibition after 7 d [%] * (growth rate based on dry weight)	0.0	0.0	0.0	0.4	0.2	1.6
Inhibition after 7 d [%] * (yield based on frond no.)	-0.8	-1.2	0.0	-1.6	-1.7	2.7
Inhibition after 7 d [%] * (yield based on dry weight)	0.0	0.1	0.1	1.3	0.7	4.6
	Endpoints [mg Peak B/L] (nominal)					
E_rC_{50} (7 d) based on frond no. and dry weight	> 100					
E_rC_{10} (7 d) based on frond no. and dry weight	> 100					
E_yC_{50} (7 d) based on frond no. and dry weight	> 100					
E_yC_{10} (7 d) based on frond no. and dry weight	> 100					

* Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} and E_yC_{50} of Peak B (metabolite of bentazone) were determined to be > 100 mg /L based on both, frond number and dry weight (nominal).

Reference number:	II A 8.6/5
Report:	Swierkot A. 2011(b) Reg.No. 5051517 (metabolite of BAS 351 H, peak C) - Lemna gibba L. CPCC 310 growth inhibition test Institute of Industrial Organic Chemistry; Pszczyna; Poland unpublished BASF DocID 2011/1124107
Guidelines:	OECD 221
GLP:	Yes (laboratory certified by Bureau for Chemical Substances and Preparations, Lodz, Poland)

Executive Summary

In a 7-day semi-static toxicity laboratory study, the effect of Peak C (metabolite of bentazone, M351H024) on the growth of the duckweed *Lemna gibba* was investigated. The following nominal concentrations were applied: 0 (control), 5.6, 10, 18, 32, 56 and 100 mg Peak C/L. Assessment of growth and other effects was conducted 2, 5 and 7 days after test initiation. The percentage growth inhibition, relative to the control, was calculated for each test concentration based upon mean growth rate data and final yield for the parameters frond number and dry weight.

The biological results are based on nominal concentrations. The duckweed population in the control vessels showed exponential growth, increasing from 9 fronds per vessel to an average of 91 fronds per vessel, corresponding to a 10 x multiplication. The dry weight increased from 1.06 mg to an average of 11.7 mg per vessel in the control at test termination. No morphological effects were observed in the control group and any test item concentration.

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} and E_yC_{50} of Peak C (metabolite of bentazone) were determined to be > 100 mg /L based on both, frond number and dry weight (nominal).

I. MATERIAL AND METHODS

Test item:	Peak C (metabolite of bentazone, Reg. No. 505 1517), batch no. L84-56, purity: 96.0%.
Test species:	Duckweed (*Lemna gibba* G3), inocula 7 day old cultures; cultures maintained in-house; stock obtained from "University of Waterloo, Canadian Phycological Culture Centre", Ontario, Canada.
Test design:	Semi-static system (7 days); renewal of test solution after 2 and 5 days; 7 treatment groups (6 test item concentrations, control) with 3 replicates for the test item treatments and 6 replicates for the control; 3 plants with 3 fronds, total number of fronds at test initiation: 9 per replicate; assessment of growth and other effects on days 2, 5 and 7.
Endpoints:	EC_{10} and EC_{50} with respect to growth rate and yield after exposure over 7 days.
Test concentrations:	Control, 5.6, 10, 18, 32, 56 and 100 mg Peak C/L (nominal).
Test conditions:	150 mL glass vessels, test volume 120 mL, 20x-AAP nutrient medium, pH 7.75 - 7.86 at test initiation and pH 9.16 - 9.37 at test termination; water temperature: 23.6 - 24.7 °C, continuous light, light intensity: 8.82 - 9.605 klux.
Analytics:	Analytical verification of the test item was conducted using a HPLC-method with UV detection.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentrations was conducted in each test concentration at the beginning and at the end of the test. Measured values for Peak C ranged from 95.32% to 97.86% of the nominal concentration at test initiation and from 98.12% to 111.96% of the nominal concentration at test termination. As analytical data confirmed generally the correct application of the test item, the following biological results are based on nominal concentrations.

Biological results: The duckweed population in the control vessels showed exponential growth, increasing from 9 fronds per vessel to an average of 91 fronds per vessel, corresponding to a 10 x multiplication. The dry weight increased from 1.06 mg to an average of 11.7 mg per vessel in the control at test termination. No morphological effects were observed in the control group and any test item concentration. Effects on growth rate and yield are summarized in Table 8.6/5.

Table 8.6/5 Effect of Peak C (metabolite of bentazone) on the growth of duckweed *Lemna gibba*

Concentration [mg/L](nominal)	**5.6**	**10**	**18**	**32**	**56**	**100**
Inhibition after 7 d [%] * (growth rate based on frond no.)	0.7	2.0	-0.3	0.1	2.2	4.8
Inhibition after 7 d [%] * (growth rate based on dry weight)	2.1	2.3	0.4	1.1	3.7	2.2
Inhibition after 7 d [%] * (yield based on frond no.)	1.6	5.3	-0.4	0.4	5.3	11.8
Inhibition after 7 d [%] * (yield based on dry weight)	5.3	6.6	1.7	3.6	9.7	6.1
	Endpoints [mg Peak C/L] (nominal)					
E_rC_{50} (7 d) based on frond no. and dry weight	> 100					
E_rC_{10} (7 d) based on frond no. and dry weight	> 100					
E_yC_{50} (7 d) based on frond no. and dry weight	> 100					
E_yC_{10} (7 d) based on frond no. and dry weight	> 100					

* Negative values indicate stimulated growth compared to the control.

III. CONCLUSION

In a 7-day aquatic plant test with *Lemna gibba* the E_rC_{50} and E_yC_{50} of Peak C (metabolite of bentazone) were determined to be > 100 mg /L based on both, frond number and dry weight (nominal).

In addition, a 14-day toxicity study on *Lemna gibba* was performed with the bentazone DEA-salt (Hughes & Alexander, 1991, BASF DocID 1991/5195) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

In the 14-day aquatic plant test with *Lemna gibba* the E_bC_{50} of bentazone was determined to be 5.35 mg /L (mean measured).

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996) and in the EC Review Report (7585/VI/97-final, November 2000) for bentazone.

Summary of results

The effects of bentazone and its major metabolites on growth of aquatic plants are summarized in Table 8.6/6.

Table 8.6/6 Summary of effects of bentazone and its major metabolites on aquatic plants

Test species	Test system	Result [mg/L]		Reference
		EC_{50}	NOEC	
Bentazone				
L. gibba	Static 7 d	25.0 [1)] 9.2 [2)]	3.3 [3)] 3.8 [4)]	Hoffmann, 2011/1102365
L. gibba [#]	Static 7 d	22.2 [1)] 9.7 [2)]	3.9 [3)] 3.4 [4)]	Hoffmann, 2011/1102366
L. gibba *	Static 14 d	5.35 [2)]	3.06	Hughes & Alexander, 1991/5195
N-methyl-bentazone (= M351H009, Reg. No. 79 520)				
L. gibba	Static 7 d	35.84 [1)] 21.32 [2)]	13.93 [3)] 9.0 [4)]	Junker, 2004/1003981
Peak B (= M351H023, Reg. No. 5 831 080)				
L. gibba	Static 7 d	> 100 [1), 2)]	> 100 [3), 4)]	Hoffmann, 2011/1165915
Peak C (= M351H024, Reg. No. 5 051 517)				
L. gibba	Static 7 d	> 100 [1), 2)]	> 100 [3), 4)]	Swierkot, 2011/1124107

[1)] E_rC_{50} [2)] E_yC_{50} / E_bC_{50} [3)] E_rC_{10} [4)] E_yC_{10} / E_bC_{10}

[#] Study was conducted with bentazone Na-salt.

[*] Study was conducted with bentazone DEA-salt.

8.7 Effects on bees

An acute oral and contact toxicity study on bees has been performed with bentazone according to recent guideline. It is reported here (see chapters 8.7.1 and 8.7.2) as it contains relevant information and was not submitted yet in an Annex II dossier.

In addition, a toxicity study on acute oral and contact toxicity on bees was performed with bentazone (Sack, 1994, BASF DocID 1994/10508 and Amendments 1994/10518 and 1997/10941) in accordance with UK working document 7/3 (1986) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

In an acute oral and contact toxicity study with bentazone on honeybees the LD_{50} values (24 h) were determined to be > 200 µg a.s./bee for oral and contact exposure.

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996) and in the EC Review Report (7585/V1197-final, November 2000) for bentazone.

8.7.1 Acute oral toxicity

Report:	II A 8.7.1/1 Bocksch S. 2003(b) Assessment of side effects of BAS 351 H to the honey bee, Apis mellifera L. in the laboratory BASF DocID 2003/1014089
Guidelines:	OECD 213; OECD 214
Deviations:	none
GLP:	Yes (laboratory certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

In an oral toxicity limit test, honeybees (young adult worker bees of *Apis mellifera* L.) were exposed to bentazone. The toxicity of the test product was determined at a nominal concentration of 150.0 µg a.s./bee; resulting in an actual uptake of 159.16 µg a.s./bee. Additionally, honeybees were treated with Perfekthion (dimethoate) as toxic reference item at concentrations ranging from 0.08 to 0.30 µg dimethoate/bee or with aqueous sugar solution or an acetone sugar solution as control or solvent control, respectively.

After 48 hours of oral exposure, no mortality was observed for water and solvent controls and 2% mortality was observed in the treatment group of 150.0 µg bentazone/bee (corresponding to an actual intake of 159.16 µg a.s./bee).

In an oral toxicity study with bentazone on honeybees the LD_{50} value (48 h) was determined to be > 159.16 µg a.s./bee.

I. MATERIAL AND METHODS

Test item:	Bentazone (BAS 351 H, Reg. no. 51 929), batch no. 14-7887, purity: 98.4% (analyzed).
Test species:	*Apis mellifera carnica* L. (honeybee), worker bees derived from a healthy colony which descended from a breeding line of a beekeeper in Rheinland-Pfalz (B. Nengel, 56348 Dahlheim, Germany); randomly collected from the outer combs of the colony the day before test start.
Test design:	In a 48 hour test, young adult worker bees of *Apis mellifera* were exposed orally to one concentration of bentazone via food (sugar syrup). In total, 7 treatment groups were set up (1 concentration of the test item, water control and solvent control and 4 concentrations of the toxic standard) with 5 replicates per treatment and 10 bees per replicate. Assessment of bee mortality and behavioral effects was done after 4, 24 and 48 hours.
Endpoint:	LD_{50}.
Toxic reference item:	Perfekthion (dimethoate, 400 g/L nominal).
Test concentrations:	Bentazone: 150.0 µg a.s./bee in an aqueous sugar solution (50% (w/v)), resulting in an uptake of 159.16 µg a.s./bee. Control (aqueous sugar solution 50% (w/v)) and a solvent control (acetone mixed with 50% (w/v) aqueous sucrose solution (1:10)). Reference item: 0.08 - 0.30 µg dimethoate/bee in an aqueous sugar solution (50% (w/v)).
Test conditions:	Temperature: 24 °C - 28 °C; relative humidity: 53% - 60%, photoperiod: 24 h darkness. Food: aqueous sucrose solution (50% (w/v)).
Statistics:	Descriptive statistics; Probit analysis for calculation of the LD_{50} value of the toxic reference item.

II. RESULTS AND DISCUSSION

After 48 hours of oral exposure, no mortality was observed for both controls and 2% mortality was observed in the treatment group of 150.0 µg bentazone/bee (corresponding to an actual intake of 159.16 µg a.s./bee). No test item induced behavioral effects were observed. The results are summarized in Table 8.7/1.

Table 8.7/1 Toxicity of bentazone to *Apis mellifera (*honeybee*)* in an oral toxicity test

Treatment	Uptake of test item	Mortality [%]	
[µg a.s./bee]	**[µg a.s./bee]**	**24 h**	**48 h**
Water control	--	0.0	0.0
Solvent control	--	0.0	0.0
150.0	159.16	0.0	2.0
Endpoint [µg a.s./bee]			
LD_{50} (48 h)	**> 159.16**		

The LD_{50} value (24 h) for the toxic reference item was 0.21 µg dimethoate/bee (95% confidence limits: 0.19 - 0.26 µg) in the oral toxicity test.

III. CONCLUSION

In an oral toxicity study with bentazone on honeybees the LD_{50} value (48 h) was determined to be > 159.16 µg a.s./bee.

8.7.2 Acute contact toxicity

Report: II A 8.7.2/1
Bocksch S. 2003(b)
Assessment of side effects of BAS 351 H to the honey bee, Apis mellifera L. in the laboratory
BASF DocID 2003/1014089
Guidelines: OECD 213; OECD 214
Deviations: none
GLP: Yes
(laboratory certified by Ministerium fuer Umwelt und Verkehr Baden-Wuerttemberg, Stuttgart)

Executive Summary

In a contact toxicity limit test, honeybees (young adult worker bees of *Apis mellifera* L.) were exposed to bentazone. The toxicity of the test product was determined at a nominal concentration of 150.0 µg a.s./bee. Additionally, honeybees were treated with Perfekthion (dimethoate) as toxic reference item at concentrations ranging from 0.11 to 0.34 µg dimethoate/bee or with water or with acetone as control or solvent control, respectively.

After 48 hours of oral exposure, no mortality was observed for both controls and 2% mortality was observed in the treatment group of 150.0 g bentazone/bee. No test item induced behavioral effects were observed at any time.

In a contact toxicity study with bentazone on honeybees the LD_{50} value (48 h) was determined to be > 150.0 µg a.s./bee.

I. MATERIAL AND METHODS

Test item: Bentazone (BAS 351 H, Reg. no. 51 929), batch no. 14-7887, purity: 98.4% (analyzed).

Test species: *Apis mellifera carnica* L. (honeybee), worker bees derived from a healthy colony which descended from a breeding line of a beekeeper in Rheinland-Pfalz (B. Nengel, 56348 Dahlheim, Germany); randomly collected from the outer combs of the colony the day before test start.

Test design: In a 48 hour test, young adult worker bees of *Apis mellifera* were exposed to one concentration of bentazone in an appropriate carrier (acetone) placed on the ventral bee thorax. In total, 7 treatment groups were set up (1 concentration of the test item, water control group, solvent control group and 4 concentrations of the toxic standard) with 5 replicates per treatment and 10 bees per replicate.
Assessment of bee mortality and behavioral effects was done after 4, 24, and 48 hours.

Endpoints LD_{50}.

Toxic reference item: Perfekthion EC (dimethoate, 400 g/L).

Test concentrations: Bentazone: 150.0 µg a.s./bee.
Control (tap water) and a solvent control (acetone).
Reference item: 0.11 - 0.34 µg dimethoate/bee.

Test conditions: Temperature: 24 °C - 28 °C; relative humidity: 53% - 60%, photoperiod: 24 h darkness. Food: aqueous sucrose solution (50% (w/v)).

Statistics: Descriptive statistics; Probit analysis for calculation of the LD_{50} value of the toxic reference item.

II. RESULTS AND DISCUSSION

After 48 hours of oral exposure, no mortality was observed for both controls and 2% mortality was observed in the treatment group of 150.0 g bentazone/bee. No test item induced behavioral effects were observed at any time. The results are summarized in Table 8.7/2.

Table 8.7/2 Toxicity of bentazone to *Apis mellifera* (honeybee) in a contact toxicity test

Treatment	Mortality [%]	
[µg a.s./bee]	**24 h**	**48 h**
Water control	0.0	0.0
Solvent control	0.0	0.0
150.0	2.0	2.0
Endpoint [µg a.s./bee]		
LD_{50} (48 h)	**> 150.0**	

The LD_{50} value (24 h) for the toxic standard was 0.18 µg dimethoate/bee (95% confidence limits: 0.16 - 0.19 µg) in the contact toxicity test.

III. CONCLUSION

In a contact toxicity study with bentazone on honeybees the LD_{50} value (48 h) was determined to be > 150.0 µg a.s./bee.

8.7.3 Toxicity of residues on foliage to honey bees

The residual toxicity of pesticides to honeybees is not an obligatory requirement according to the EPPO risk assessment scheme and Commission regulation (EU) 546/2011. The risk to honeybees is fully covered by the studies submitted under Annex II and Annex III. Hence, no residue study is required.

8.7.4 Bee brood feeding test

Bentazone is not an insect growth regulator. According to Commission regulation (EU) 546/2011 no bee brood study is required. The risk to honeybees will be fully covered by the studies submitted under Annex II and Annex III.

Summary of results

The effects of bentazone on bees are summarized in Table 8.7/3.

Table 8.7/3 Summary of data for bentazone on *Apis mellifera* (honeybee)

Test Item	Test System	LD_{50} [µg/bee]	Reference
Bentazone [1)]	Acute oral	> 200.0	Sack, 1994/10508
	Acute contact	> 200.0	
Bentazone [2)]	Acute oral	> 159.16	Boksch, 2003/1014089
	Acute contact	> 150.0	

1) Study conducted in accordance with UK working document 7/3 (1986)
2) Study conducted in accordance with recent guideline (OECD 213 and 214)

8.8 Effects on non-target terrestrial arthropods

8.8.1 Effects on non-target terrestrial arthropods, artificial substrates

8.8.1.1 Parasitoid

A worst-case laboratory study on *Trichogramma cacoeciae* was performed with BAS 351 45 H (containing 87% bentazone); Kuehner, 1994, BASF DocID 1994/10242 and Amendments 1994/10594 and 1994/10796) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

Under worst-case laboratory conditions, unacceptable effects of 51.3% were observed on parasitic capacity of *Trichogramma cacoeciae* at an application rate of 3.0 kg BAS 351 45 H/ha.

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996) and in the EC Review Report (7585/V1197-final, November 2000) for bentazone.

For further testing of parasitoids on inert substrate please refer to chapter MIII, 10.5.

8.8.1.2 Predatory mites

For further testing of predatory mite species on inert substrate please refer to chapter MIII, 10.5.

8.8.1.3 Ground dwelling predatory species

A worst-case laboratory study on *Aleochara bilineata* was performed with BAS 351 32 H (= Basagran; containing nominal 480 g/L bentazone); Samsoe-Petersen, 1990, BASF DocID 1990/10104) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

Under worst-case laboratory conditions, no unacceptable effects on reproduction of *Aleochara bilineata* were observed at an application rate of 2.4 L BAS 351 32 H/ha.

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996) and in the EC Review Report (7585/V1197-final, November 2000) for bentazone.

A worst-case laboratory study on *Poecilus cupreus* was performed with BAS 351 45 H (containing 87% bentazone); Schlosser, 1994, BASF DocID 1994/10473) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

Under worst-case laboratory conditions, no unacceptable effects on survival and feeding activity of *P. cupreus* were observed at an application rate of 3.0 kg BAS 351 45 H/ha.

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996) and in the EC Review Report (7585/V1197-final, November 2000) for bentazone.

For further testing of ground dwelling predatory species on inert substrate please refer to chapter MIII, 10.5.

A worst-case laboratory study on *Bembidion lampros* was performed with BAS 351 32 H (= Basagran; containing nominal 480 g/L bentazone); Kuenast, 1989, BASF DocID 1989/10804) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

Under worst-case laboratory conditions, no unacceptable effects on survival *of Bembidion lampros* were observed at an application rate of 4.0 L BAS 351 32 H/ha.

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996) and in the EC Review Report (7585/V1197-final, November 2000) for bentazone.

8.8.1.4 Foliage dwelling predatory species

A worst-case laboratory study on *Chrysoperla carnea* was performed with BAS 351 45 H (containing 87% bentazone); Kuenast, 1993, BASF DocID 1993/11269) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

Under worst-case laboratory conditions, no unacceptable effects on survival and reproduction of *C. carnea* were observed at an application rate of 3.0 kg BAS 351 45 H/ha.

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996) and in the EC Review Report (7585/V1197-final, November 2000) for bentazone.

For further testing of foliage dwelling predatory species on inert substrate please refer to chapter MIII, 10.5.

8.8.2 Effects on non-target terrestrial arthropods in lab/semi-field test

8.8.2.1 Parasitoid

For further testing of parasitoid species on natural substrate please refer to chapter MIII, 10.5.

8.8.2.2 Predatory mites

For further testing of predatory mite species on natural substrate please refer to chapter MIII, 10.5.

8.8.2.3 Ground dwelling predatory species

Further testing of ground dwelling predatory species on natural substrate is not required.

8.8.2.4 Foliage dwelling predatory species

For further testing of foliage dwelling predatory species on natural substrate please refer to chapter MIII, 10.5.

8.8.2.5 Other terrestrial invertebrates

Toxicity testing on other terrestrial invertebrates, such as honeybees and earthworms, are described in Document M-II, chapters 8.7 and 8.9, respectively (for the active ingredient bentazone) and in Document M-III, chapter 10.4 and 10.6, respectively (for the formulation BAS 351 32 H).

Summary of results

The effects of BAS 351 32 H and BAS 351 45 H on non-target arthropods are summarized in Table 8.8/1.

Table 8.8/1 Summary of effects of BAS 351 32 H and BAS 351 45 H on non-target arthropods

Test species	Study type	LR_{50}	Sublethal effects	Reference
BAS 351 45 H				
Trichogramma cacoeciae	Laboratory test using artificial substrate	--	unacceptable effects of 51.3% on parasitic capacity at 3.0 kg/ha	Sipos, 1994/10242 + Amendments 1994/10594 and 1994/10796
Poecilus cupreus	Laboratory test using artificial substrate	LR50 > 3.0 kg/ha	no unacceptable effects on reproduction at 3.0 kg/ha	Schlosser, 1994/10473
Chrysoperla carnea	Laboratory test using artificial substrate	LR50 > 3.0 kg/ha	no unacceptable effects on reproduction at 3.0 kg/ha	Kuenast, 1993/11269
BAS 351 32 H				
Aleochara bilineata	Laboratory test using artificial substrate	--	no unacceptable effects on reproduction at 2.4 L/ha	Samsoe-Petersen, 1990/10104
Bembidion lampros	Laboratory test using artificial substrate	no unacceptable effects on survival at 4.0 L/ha	--	Kuenast, 1989/10804

8.9 Effects on earthworms

8.9.1 Acute toxicity to earthworms

Report:	II A 8.9.1/1 Fleischer G. 2003(b) Effect of BAS 351 H (Reg.No. 51926) on the mortality of the earthworms Eisenia fetida BASF DocID 2003/1001066
Guidelines:	OECD 207
Deviations:	none
GLP:	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

In an acute toxicity study, adults of *Eisenia fetida* (Annelida: Oligochaeta), were exposed to bentazone. The test item was mixed into artificial soil at concentrations of 197.5, 296.3, 444.4, 666.7 and 1000.0 mg/kg dry soil. For the control treatment, the soil was left untreated. The artificial test soil had an organic content of 10%.

After 14 days of exposure, no mortality was observed in the control and all test item concentrations. All treatment groups including the control showed a reduction in biomass. Statistically significant differences compared to the control were observed in all test item treatment groups, but at the three lowest concentrations the biomass is considered not be adversely effected since reduction rates are below 20%. No behavioral or morphological changes were observed.

In a 14-day acute toxicity study with bentazone on earthworms (*Eisenia fetida*), the LC_{50} was > 1000 mg/kg dry soil.

I. MATERIAL AND METHODS

Test item:	Bentazone (BAS 351 H, Reg. no. 51 929), batch no. 14-7887, purity: 98.4%.
Test species:	*Eisenia fetida* (earthworm); source: in-house culture, adult worms with clitellum and weight between 250 mg - 600 mg, age: less than 1 year old.
Test design:	In a 14-day test, adults of *Eisenia fetida* were exposed to five concentrations of bentazone in artificial soil. The artificial soil was according to OECD 207 (10% peat). In total, 6 treatment groups were set up (5 concentrations of the test item and untreated control group) with 4 replicates per treatment and 10 adult worms per replicate. The artificial soil was treated and filled into glass vessels before the earthworms were introduced on the top of the soil. Assessment of adult earthworm mortality was done after 7 and 14 days, behavioural effects and weight changes of the worms were assessed as sublethal parameter after 14 days.
Endpoint:	LC_{50}, (NOAEC).
Reference item:	Chloroacetamide. The effects of the reference item were investigated in a separate study.
Test concentrations:	Control, 197.5, 296.3, 444.4, 666.7, 1000.0 mg bentazone/kg dry soil.
Test conditions:	Artificial soil according to OECD 207 (content of peat of 10%); pH 6.2; water content: 18.6 g/100 g soil dry weight at test initiation, 18.1 g /100 g soil dry weight at test termination; temperature: 20 °C - 21 °C; constant illumination.
Statistics:	Descriptive statistics, Dunnett-test for weight change (α = 0.05).

II. RESULTS AND DISCUSSION

After 14 days of exposure, no mortality was observed in the control and all test item concentrations. All treatment groups including the control showed a reduction in biomass. Statistically significant differences compared to the control were observed in all test item treatment groups (Dunnett-test, α = 0.05), but at the three lowest concentrations the biomass is considered not be adversely effected since reduction rates are below 20%. No behavioral or morphological changes were observed. The results are summarized below [see Table 8.9/1].

Table 8.9/1 Effects of bentazone on survival and biomass of *Eisenia fetida* (14 d)

Bentazone [mg/kg dry soil]	**Control**	**197.5**	**296.3**	**444.4**	**666.7**	**1000**
Mortality [%]	0.0	0.0	0.0	0.0	0.0	0.0
Weight change [%]	-7.69	-14.65 *	-15.40 *	-15.26 *	-21.79 *	-31.49 *
Endpoints [mg/kg dry soil]						
NOAEC	444.4					
LC_{50}	> 1000					

* = statistically significant differences compared to the control (Dunnett-test for weight data; α = 0.05)

III. CONCLUSION

In a 14-day acute toxicity study with bentazone on earthworms (*Eisenia fetida*), the LC_{50} was > 1000 mg/kg dry soil.

Report: II A 8.9.1/2
Gehrig M. 2011(a)
Acute toxicity of BAS 351 H (Bentazon-Na, Reg.No. 88691) on earthworms, Eisenia fetida in artificial soil with 5% peat
BASF DocID 2011/1000321

Guidelines: OECD 207 (1984); ISO 11268-1 (1993)
Deviations: none

GLP: Yes
(laboratory certified by Landesamt fuer Umwelt, Messungen und Naturschutz Baden Wuerttemberg, Karlsruhe, Germany)

Executive Summary

In an acute toxicity study, adults of *Eisenia fetida* (Annelida: Oligochaeta), were exposed to bentazone-Na. The test item was mixed into artificial soil at concentrations of 198, 296, 444, 667 and 1000 mg/kg dry soil. For the control treatment, the soil was left untreated. The artificial test soil had an organic content of 5% (as sphagnum peat).

After 14 days of exposure, no mortality was observed in the control and all test item concentrations. Body weight changes were not statistically significant different compared to the control in any of the test item concentrations (Bonferroni-U-Exact Test, α = 0.05). No behavioral changes were observed.

In a 14-day acute toxicity study with bentazone-Na on earthworms (*Eisenia fetida*), the LC_{50} was > 1000 mg/kg dry soil.

I. MATERIAL AND METHODS

Test item: Bentazone-Na (BAS 351 H-Na, Reg. no. 88 691), batch no. COD-001417, purity: 91.9%.

Test species: *Eisenia fetida* (earthworm); source: in-house culture, adult worms with clitellum and weight of 320 mg - 465 mg, age: between 6 and 7 month.

Test design: In a 14-day test, adults of *Eisenia fetida* were exposed to five concentrations of bentazone-Na in artificial soil. The artificial soil was according to OECD 207 (with a reduced content of peat (5%)). In total, 6 treatment groups were set up (5 concentrations of the test item and untreated control group) with 4 replicates per treatment and 10 adult worms per replicate. The artificial soil was treated and filled into glass vessels before the earthworms were introduced on the top of the soil.
Assessment of adult earthworm mortality and behavioural effects was done after 7 and 14 days, weight changes of the worms were assessed as sublethal parameter after 14 days.

Endpoint: LC_{50}, (NOAEC).

Reference item: 2-Chloroacetamide. The effects of the reference item were investigated in a separate study.

Test concentrations: Control, 198, 296, 444, 667, 1000 mg bentazone-Na/kg dry soil.

Test conditions: Artificial soil according to OECD 207 (content of peat of 5%); pH 6.20 - 6.45 at test initiation, pH 6.63 - 6.85 at test termination;
water content 55.5% - 56.6% of the max. WHC at test initiation and 51.4% - 54.7% of the max. WHC at test termination; temperature: 18.5 °C - 19.5 °C; constant illumination at 520 lux.

Statistics: Descriptive statistics, Bonferroni-U-Exact Test, two tailed (α = 0.05).

II. RESULTS AND DISCUSSION

After 14 days of exposure, no mortality was observed in the control and all test item concentrations. Body weight changes were not statistically significant different compared to the control in any of the test item concentrations (Bonferroni-U-Exact Test, α = 0.05). No behavioral changes were observed. The results are summarized in Table 8.9/2.

Table 8.9/2 Effects of bentazone-Na on survival and biomass of *Eisenia fetida* (14 d)

Bentazone-Na [mg/kg dry soil]	**Control**	**198**	**296**	**444**	**667**	**1000**
Mortality [%]	0.0	0.0	0.0	0.0	0.0	0.0
Weight change [%]	-16.3	-18.3 n.s.	-10.3 n.s.	-13.6 n.s.	-15.9 n.s.	-22.5 n.s.
Endpoints [mg/kg dry soil]						
NOEC	≥ 1000					
LC_{50}	> 1000					

n.s. = statistically significant differences compared to the control (Bonferroni-U-Exact Test, two-tailed; α = 0.05)

III. CONCLUSION

In a 14-day acute toxicity study with bentazone-Na on earthworms (*Eisenia fetida*), the LC_{50} was > 1000 mg/kg dry soil.

Report:	II A 8.9.1/3 Fleischer G. 2004(b) Effect of BH 351-N-Me (Reg.No. 79520; metabolite of Bentazon) on the mortality of the earthworm Eisenia fetida BASF DocID 2004/1003959
Guidelines:	ISO 11268-1 (1993) **Deviations: none**
GLP:	Yes (laboratory certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)

Executive Summary

Adult earthworms of the species *Eisenia fetida* were exposed to N-methyl-bentazone (M351H009). The test item was mixed with artificial soil at concentrations of 131.7, 197.5, 296.3, 444.4, 666.7 and 1 000 mg N-methyl-bentazone/kg dry soil. For the control treatment, the soil was left untreated. Four replicates were prepared for each treatment group and the control, each containing 10 worms. Assessment of mortality and behavioral effects were made 7 and 14 days after treatment. Assessment of worm weight and behavioral impairments was made after 14 days.

After 14 days of exposure, no mortality was observed in any of the treatment groups. All treatment groups including the control showed reduction in biomass, statistically significant differences were determined at test concentrations of 444.4 mg/kg dry soil up to 1 000 mg/kg dry soil. No particular behavioral abnormalities could be observed.

In a 14-day toxicity study on earthworms (*Eisenia fetida)* with N-methyl-bentazone (M351H009), the LC_{50} was > 1 000 mg test item/kg dry soil. The NOEC with respect to mortality and biomass was 296.3 mg N-methyl-bentazone/kg dry soil.

I. MATERIAL AND METHODS

Test item:	N-methyl-bentazone (M351H009), batch No. 2235-09, purity: 99.8%.
Test species:	Earthworm (*Eisenia fetida)*, adult worms (with clitellum), age: less than one year old; weight between 200 and 600 mg; source: in-house culture.
Test design:	14-d exposure in treated artificial soil; 7 concentration groups including a control group with 4 replicates each and 10 worms per replicate; worms were placed in 1 L glass jars filled with treated and mixed artificial soil. Assessment of mortality after 7 and 14 days, assessment of weight and changes in behavior after 14 days.
Endpoints:	LC_{50} (50% mortality of earthworms after exposure over 14 days), behavioral effects, weight change.
Test concentrations:	Control, 131.7, 197.5, 296.3, 444.4, 666.7 and 1 000 mg N-methyl-bentazone/kg dry soil.
Reference item:	Chloracetamine; effects have been evaluated in a separate study.
Test conditions:	Artificial soil according to ISO 11268-1 (peat content 10%); temperature: 20 ± 2 °C; pH 6.1; water content: at test initiation 27.6 % (of soil dry weight), at test termination 25.6%; photoperiod 16 h light : 8 h dark; light intensity 400 – 800 lux.
Statistics:	Descriptive statistics for mortality, determination of NOEC (biomass) by Dunnett's t-test (α = 0.05).

II. RESULTS AND DISCUSSION

As N-methyl-bentazone caused no mortality even in the highest treatment group, the LC_{50} was determined to be > 1 000 mg test item/kg dry soil.
All treatment groups including the control showed reduction in biomass, statistically significant differences were determined at test concentrations of 444.4 mg/kg dry soil up to 1 000 mg/kg dry soil. No particular behavioral abnormalities could be observed. The results are summarized in Table 8.9/3.

Table 8.9/3 Effects of N-methyl-bentazone on earthworm (*Eisenia fetida)* mortality and biomass (14 d)

N-methyl-bentazone [mg/kg dry soil]	**Control**	**131.7**	**197.5**	**296.3**	**444.4**	**666.7**	**1000**
Mortality [%]	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Weight change [%]	-4.85	-8.35	-6.61	-7.71	-10.54*	-15.28*	-21.27*
Endpoints [mg/kg dry soil]							
NOEC	296.3						
LC_{50}	> 1 000						

* Significantly different from control, Dunnett's t-test (α = 0.05).

III. CONCLUSION

In a 14-day toxicity study on earthworms (*Eisenia fetida)* with N-methyl-bentazone (M351H009), the LC_{50} was > 1 000 mg test item/kg dry soil. The NOEC with respect to mortality and biomass was 296.3 mg N-methyl-bentazone/kg dry soil.

In addition, a 14-day acute toxicity study on earthworms (*Eisenia fetida*) was performed with bentazone (Adolphi, 1985, BASF DocID 1985/10056) and was assessed in the EU review of bentazone. It has already been submitted and accepted during Annex I evaluation for bentazone (Monograph, Vol. 3, Annex B.8, September 1996).

In the 14-day toxicity study on earthworms (*Eisenia fetida*) with bentazone, the LC_{50} was > 1 000 mg bentazone/kg dry soil.

The study is acceptable, as also stated in the Monograph (Vol. 3, Annex B.8, September 1996) for bentazone.

8.9.2 Sublethal effects on earthworms

Report:	II A 8.9.2/1 Friedrich S. 2004(b) Reg.No. 79520 (BH 351-N-Me, metabolite of BAS 351 H, Bentazon): Sublethal toxicity to the earthworm Eisenia fetida in artificial soil with 5% peat BASF DocID 2004/1025198
Guidelines:	ISO 11268-2 (1998)
Deviations:	none
GLP:	Yes (laboratory certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

Adult earthworms of the species *Eisenia fetida* were exposed to N-methyl-bentazone (M351H009). The test item was mixed with artificial soil at concentrations of 0.35, 1.75, 3.5, 7.0 and 14.0 mg N-methyl-bentazone/kg dry soil. For the control treatment, the soil was left untreated. Four replicates were prepared for each treatment group and the control, each containing 10 worms. Assessment of adult mortality and weight change was conducted after 28 days, counting of number of juveniles after 56 days.

In the N-methyl-bentazone treatment groups the biomass increase of adult worms was about 33% to 38%, the mean number of juveniles ranged from 66.8 to 93.5 in the treated variants and was 84.3 in the control group. No statistically significant differences were determined at any test concentration when compared to the control.

In a 56-d sublethal toxicity study on earthworms (*Eisenia andrei)* with N-methyl-bentazone (M351H009), no statistically significant sublethal effects of N-methyl-bentazone on adult mortality, biomass change and number of juveniles were determined. The NOEC therefore was 14.0 mg N-methyl-bentazone/kg dry soil.

I. MATERIAL AND METHODS

Test item:	N-methyl-bentazone (M351H009), batch No. 2235-09, purity: 99.8%.
Test species:	Earthworm (*Eisenia andrei)*, adult worms (with clitellum), age: about 4 months; source: in-house culture.
Test design:	56-d exposure in treated artificial soil; 5 concentration groups including a control group with 4 replicates each and 10 worms per replicate; worms were placed in plastic vessels filled with treated and mixed artificial soil. Assessment of adult mortality and weight change after 28 days, counting of number of juveniles and behavior after 56 days.
Endpoints:	LC_{50} (50% mortality of adult earthworms after 28 days), biomass change of surviving adult earthworms, surviving juveniles, behavioral effects (feeding activity).
Test concentrations:	Control, 0.35, 1.75, 3.5, 7.0 and 14.0 mg N-methyl-bentazone/kg dry soil.
Reference item:	DuPont Benomyl® WP 50 (benomyl 500 g/kg); reference item was tested in separate study.
Test conditions:	Artificial soil according to ISO 11268-2 (peat content 5%); temperature: 19 - 22 °C; pH 6.33 – 6.46 (beginning) and 6.38 – 6.47 (test termination); water content: at test initiation 25.1 – 25.5% (of soil dry weight), at test termination 24.9 – 25.5%; photoperiod 16 h light : 8 h dark; light intensity 600 lux; food: horse manure.
Statistics:	Descriptive statistics for mortality, determination of biomass and reproduction by Dunnett's t-test (α = 0.05).

II. RESULTS AND DISCUSSION

N-methyl-bentazone caused no mortality in any test item treatment group.
In the N-methyl-bentazone treatment groups the biomass increase of adult worms was about 33% to 38%, the mean number of juveniles ranged from 66.8 to 93.5 in the treated variants and was 84.3 in the control group. No statistically significant differences were determined at any test concentration (Dunnett's t-test, α = 0.05). The results are summarized in Table 8.9/4.

Table 8.9/4 Effects of N-methyl-bentazone on earthworm (*Eisenia andrei)* mortality, biomass and reproduction (56 d)

N-methyl-bentazone [mg/kg dry soil]	**Control**	**0.35**	**1.75**	**3.5**	**7.0**	**14.0**
Mortality [%]	0.0	0.0	0.0	0.0	0.0	0.0
Weight change [%]	+36.3	+38.4	+34.9	+37.4	+37.6	+33.3
Number of juveniles [% of control]	-	96.7	111.0	100.6	82.2	79.2
Amount of food added [g/vessel]	30.6	30.6	30.6	30.6	30.6	30.6
Endpoints [mg/kg dry soil]						
NOEC	≥ 14.0					
LC_{50}	> 14.0					

III. CONCLUSION

In a 56-d sublethal toxicity study on earthworms (*Eisenia andrei)* with N-methyl-bentazone (M351H009), no statistically significant sublethal effects of N-methyl-bentazone on adult mortality, biomass change and number of juveniles were determined. The NOEC therefore was 14.0 mg N-methyl-bentazone/kg dry soil.

Summary of results

The effects of bentazone, bentazone-Na and metabolite N-methyl-bentazone on earthworms are summarized in Table 8.9/5.

Table 8.9/5 Summary of effects of bentazone, bentazone-Na and N-methyl-bentazone on earthworms

Test species	Test system	Toxicity [mg/kg dry soil]		Reference
		LC_{50} / EC_{50}	NOEC	
bentazone				
E. fetida	14-d toxicity test	> 1000	444.4	Friedrich, 2003/1001066
E. fetida [2)]	14-d toxicity test	> 1000	≥ 1000	Adolphi, 1985/10056
bentazone-Na				
E. fetida	14-d toxicity test [1)]	> 1000	≥ 1000	Gehrig, 2011/1000321
N-methyl-bentazone				
E. fetida	14-d toxicity test	> 1000	296.3	Fleischer, 2004/1003959
E. fetida	56-d reproduction test [1)]	> 14.0	≥ 14.0	Friedrich, 2004/1025198

[1)] Test substrate contained 5% peat.
[2)] Non-GLP study.

8.10 Effects on soil microbial activity

8.10.1 Nitrogen transformation

Report:	II A 8.10.1/1 Schulz L. 2011(b) Effects of Reg.No. 51929 on the activity of soil microflora (nitrogen transformation test) BASF DocID 2010/1144223
Guidelines:	OECD 216 (2000)
Deviations:	None
GLP:	Yes (laboratory certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a soil microbial activity study, the effects of bentazone on the nitrogen transformation were investigated in a lucerne-enriched silty sand soil. Bentazone was applied to samples of the soil at nominal concentrations of 1.92 and 5.76 mg/kg dry soil. Triplicate samples of each treatment were removed for analysis of NO_3 content 0, 7, 14 and 28 days after application and in the higher test concentration additionally on days 42 and 56.

No unacceptable effects of bentazone on nitrogen transformation were observed after 28 days in the treatment group of 1.92 mg bentazone/kg dry soil. The deviation from control was 19.8% after 28 days. In the treatment group of 5.76 mg bentazone/kg dry soil deviations from control were > 25% after 28 days and 42 days. At the end of the 56-day incubation period the deviation from the control was +13.8%.

Based on the results of this study, bentazone caused no long-term effects on the soil nitrogen transformation (measured as NO_3 production) in a silty sand soil tested up to a concentration of 5.76 mg bentazone/kg dry soil.

I. MATERIAL AND METHODS

Test item:	Bentazone (BAS 351 H, Reg. no. 51 929), batch no. COD-001416, purity: 100.0% (tolerance ± 1.0%).
Test soil:	Biologically active agricultural soil: silty sand soil (DIN 4220); pH 6.4, 1.42% C_{org} and 37.18% WHC.
Test design:	Determination of the N-transformation (NO_3 production) in soil enriched with lucerne meal resulting in a soil concentration of 0.5%. Three treatment groups were set up (one untreated control group and two concentrations of the test item) with three replicates per treatment. NH_4–nitrogen formed from organically bound nitrogen in the soil and NO_3–nitrogen from the nitrification process was determined using an Autoanalyzer II (Bran and Luebbe). Sampling scheme: 0, 7, 14, 28, 42 and 56 days after treatment. Sub-samples were withdrawn from the bulk batches and subjected to measurement.
Endpoint:	Concentration producing maximum 25% deviation considering NO_3 production compared to the control.
Reference item:	Dinoterb (purity: 98% ± 0.5%). The reference item was tested in a separate study at rates of 6.8, 16.0 and 27.0 mg Dinoterb/kg dry soil.
Test concentrations:	Control, 1.92 mg bentazone/kg dry soil and 5.76 mg bentazone/kg dry soil.
Test conditions:	Soil moisture: approx. 45% of its water holding capacity corresponding to a measured water content of 17.07% - 18.55%, pH 6.2 - 6.4. Soil samples were incubated at 18.6 °C - 21.2 °C while stored in glass flasks in the dark.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

No unacceptable effects of bentazone on nitrogen transformation were observed after 28 days in the treatment group of 1.92 mg bentazone/kg dry soil. The deviation from control was 19.8% after 28 days. In the treatment group of 5.76 mg bentazone/kg dry soil deviations from control were > 25% after 28 days and 42 days. At the end of the 56-day incubation period the deviation from the control was +13.8%. The results are summarized in Table 8.10/1.

Table 8.10/1 Effects of bentazone on soil micro-organisms (nitrogen transformation) on days 7, 14, 28, 42 and 56 of incubation

Soil (days)	Control	1.92 mg bentazone/kg dry soil		5.76 mg bentazone/kg dry soil	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]
Silty sand soil (7 d)	14.53	16.10	+10.8	19.70	+35.6
Silty sand soil (14 d)	18.20	22.97	+26.2	29.23	+60.6
Silty sand soil (28 d)	29.50	35.33	+19.8	39.50	+33.9
Silty sand soil (42 d)	41.13	--*	--	52.33	+27.2
Silty sand soil (56 d)	64.53	-- *	--	73.43	+13.8

[1)] Based on NO_3-N production; - = inhibition; + = stimulation.
* No analysis was performed.

In a separate study, the reference item Dinoterb caused effects on the N-transformation of +42.0%, +68.1% and +92.3% at test concentrations of 6.8, 16.0 and 27.0 mg Dinoterb/kg dry soil, respectively.

III. CONCLUSION

Based on the results of this study, bentazone caused no long-term effects on the soil nitrogen transformation (measured as NO_3 production) in a silty sand soil tested up to a concentration of 5.76 mg bentazone/kg dry soil.

Report:	II A 8.10.1/2 Schulz L. 2011(c) Effects of Reg.No. 88691 on the activity of soil microflora (nitrogen transfomation test) BASF DocID 2011/1057030
Guidelines:	OECD 216 (2000)
Deviations:	None
GLP:	Yes (laboratory certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a soil microbial activity study, the effects of bentazone-Na on the nitrogen transformation were investigated in a lucerne-enriched silty sand soil. Bentazone-Na was applied to samples of the soil at nominal concentrations of 2.09 and 6.27 mg/kg dry soil. Triplicate samples of each treatment were removed for analysis of NO_3-N content 0, 7, 14, 28 and 42 days after application.

No unacceptable effects of bentazone-Na on nitrogen transformation were observed after 42 days in the treatment group of 2.09 mg bentazone-Na/kg dry soil and 6.27 mg bentazone-Na/kg dry soil. The deviation from control were +15.4% and +24.6% after 42 days, respectively.

Based on the results of this study, bentazone-Na caused no long-term effects on the soil nitrogen transformation (measured as NO_3-N production) in a silty sand soil tested up to a concentration of 6.27 mg bentazone-Na/kg dry soil.

I. MATERIAL AND METHODS

Test item:	Bentazone-Na (BAS 351 H-Na, Reg. no. 88 691), batch no. COD-001417, purity: 91.9% (tolerance ± 1.0%).
Test soil:	Biologically active agricultural soil: silty sand soil (DIN 4220); pH 6.4, 1.42% C_{org} and 37.18% WHC.
Test design:	Determination of the N-transformation (NO_3–nitrogen production) in soil enriched with lucerne meal resulting in a soil concentration of 0.5%. Three treatment groups were set up (one untreated control group and two concentrations of the test item) with three replicates per treatment. NH_4–nitrogen formed from organically bound nitrogen in the soil and NO_3–nitrogen from the nitrification process was determined using an Autoanalyzer II (Bran and Luebbe). Sampling scheme: 0, 7, 14, 28 and 42 days after treatment. Sub-samples were withdrawn from the bulk batches and subjected to measurement.
Endpoint:	Concentration producing maximum 25% deviation considering NO_3 production compared to the control.
Reference item:	Dinoterb (purity: 98% ± 0.5%). The reference item was tested in a separate study at rates of 6.8, 16.0 and 27.0 mg Dinoterb/kg dry soil.
Test concentrations:	Control, 2.09 mg bentazone-Na/kg dry soil and 6.27 mg bentazone-Na/kg dry soil.
Test conditions*:*	Soil moisture: approx. 45% of its water holding capacity corresponding to a measured water content of 17.39% - 18.29%, pH 6.2 - 6.4. Soil samples were incubated at 19.3 °C - 21.2 °C while stored in glass flasks in the dark.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

No unacceptable effects of bentazone-Na on nitrogen transformation were observed after 42 days in the treatment group of 2.09 mg bentazone-Na/kg dry soil and 6.27 mg bentazone-Na/kg dry soil. The deviation from control were +15.4% and +24.6% after 42 days, respectively. The results are summarized in Table 8.10/2.

Table 8.10/2 Effects of bentazone-Na on soil micro-organisms (nitrogen transformation) on days 7, 14, 28 and 42 of incubation

Soil (days)	Control	2.09 mg bentazone-Na/kg dry soil		6.27 mg bentazone-Na/kg dry soil	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]
Silty sand soil (7 d)	14.53	17.87	+22.9	23.20	+59.6
Silty sand soil (14 d)	18.20	25.90	+42.3	32.50	+78.6
Silty sand soil (28 d)	29.50	37.70	+27.8	44.53	+51.0
Silty sand soil (42 d)	41.13	47.47	+15.4	51.23	+24.6

[1)] Based on NO_3-N production; - = inhibition; + = stimulation

In a separate study, the reference item Dinoterb caused effects on the N-transformation of +42.0%, +68.1% and +92.3% at test concentrations of 6.8, 16.0 and 27.0 mg Dinoterb/kg dry soil, respectively.

III. CONCLUSION

Based on the results of this study, bentazone-Na caused no long-term effects on the soil nitrogen transformation (measured as NO_3-N production) in a silty sand soil tested up to a concentration of 6.27 mg bentazone-Na/kg dry soil.

Report: II A 8.10.1/3
Schulz L. 2004(c)
Effects of Reg.No. 79520 (BH 351-N-ME, metabolite of BAS 351 H, Bentazon) on the activity of soil microflora (Nitrogen transformation test)
BASF DocID 2004/1014995

Guidelines: OECD 216 (2000)

Deviations: None

GLP: Yes
(laboratory certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a soil microbial activity study, the effects of N-methyl-bentazone (M351H009) on the nitrogen transformation were investigated in a sandy loam. N-methyl-bentazone was applied to samples of the soil at 0.4 and 2.0 mg N-methyl-bentazone/kg dry soil, corresponding to 0.275 kg test item/ha and 1.375 kg test item/ha, respectively.

There were no significant effects on the rate of conversion of NH_4-N to NO_3-N at any application rate.

N-methyl-bentazone (M351H009) caused no adverse effects (deviation from control <25%, according to current OECD 216 guideline) on the soil N-transformation (measured as NO_3-N production) at the end of the incubation period. The study was performed in a sandy loam field soil at concentrations up to 2.0 mg N-methyl-bentazone/kg dry soil, corresponding to 1.375 kg test item/ha.

I. MATERIAL AND METHODS

Test item:	N-methyl-bentazone (M351H009), batch No. 2235-09, purity: 99.8%.
Test species:	Biologically active agricultural soil: sandy loam soil; pH 6.9, 1.49% C_{org}, 44.5% WHC, 11.3 cmol/kg dry soil CEC.
Test design:	Determination of the nitrogen transformation (NO_3-nitrogen production) in soil enriched with Lucerne meal (concentration in soil 0.5%). Comparison of test item treated soil with a non-treated soil. 3 replicates per treatment and concentration. NH_4-nitrogen formed from organically bound nitrogen in the soil, NO_3- and NO_2-nitrogen from the nitrification process was determined using an Autoanalyzer II (Bran+Luebbe). Sampling scheme: 0, 7, 14 and 28 days after treatment; sub-samples were withdrawn from the bulk batches and subjected to measurement.
Endpoints:	Effects on the NO_3-nitrogen production in sandy loam soil after 28 days of exposure.
Test concentrations:	Control, 0.4 and 2.0 mg N-methyl-bentazone/kg dry soil, corresponding to 0.275 kg test item/ha and 1.375 kg test item/ha, respectively. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g dry soil/cm^3.
Reference item:	Dinosep acetate, 8.67 mg/kg dry soil; effects have been evaluated in a separate study.
Test conditions:	soil moisture: 45% of soil maximum water holding capacity. Soil samples were incubated at 20 °C ± 2 °C while stored in new plastic vessels.
Statistics:	Mean values per treatment, standard deviations and coefficients of variation.

II. RESULTS AND DISCUSSION

No adverse effects of N-methyl-bentazone on nitrogen transformation in soil were observed at both application rates after 28 days. Only slight deviations from the control of -1.3% and -0.7% were measured in the concentrations in the sandy loam soil at day 28. The results are summarized in Table 8.10/3.

Table 8.10/3 Effects of N-methyl-bentazone on soil micro-organisms (nitrogen transformation) after 28 days of incubation

Soil (days)	Control	0.4 mg test item/kg dry soil		2.0 mg test item/kg dry soil	
	NO_3-N [mg/kg dry soil]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]	NO_3-N [mg/kg dry soil]	% Deviation from control [1)]
Sandy loam soil (7 d)	51.1	51.9	+1.4	51.4	+0.5
Sandy loam soil (14 d)	60.7	59.3	-2.4	57.0	-6.1
Sandy loam soil (28 d)	71.7	70.8	-1.3	71.2	-0.7

[1)] Based on NO_3-nitrogen production; - = inhibition; + = stimulation

The reference item produced the expected level of effect (27.7% increase of nitrogen transformation on day 28).

III. CONCLUSION

N-methyl-bentazone (M351H009) caused no adverse effects (deviation from control <25%, according to current OECD 216 guideline) on the soil N-transformation (measured as NO_3-N production) at the end of the incubation period. The study was performed in a sandy loam field soil at concentrations up to 2.0 mg N-methyl-bentazone/kg dry soil, corresponding to 1.375 kg test item/ha.

8.10.2 Carbon mineralization

Report:	II A 8.10.2/1 Schulz L. 2011(a) Effects of BAS 351 H (Reg.No. 51929) on the activity of soil microflora (carbon transformation test) BASF DocID 2010/1144219
Guidelines:	OECD 217 (2000)
Deviations:	None
GLP:	Yes (laboratory certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

In a soil microbial activity study, the effects of bentazone, on the carbon transformation were investigated in a loamy sand soil. Bentazone was applied to samples of the soil at nominal concentrations of 1.92 and 19.20 mg/kg dry soil. Triplicate samples of each treatment were removed for analysis of carbon transformation (oxygen consumption) 7, 14 and 28 days after application.

No significant influences of bentazone on the carbon transformation were observed after 28 days, only slight deviations from the control of -2.2% and -23.2% were measured at the concentrations of 1.92 and 19.20 mg/kg dry soil.

Based on the results of this study, bentazone caused no short-term and no long-term effects on the carbon transformation (measured as oxygen consumption) in a loamy sand field soil tested up to a concentration of 19.20 mg bentazone/kg dry soil.

I. MATERIAL AND METHODS

Test item:	Bentazone (BAS 351 H, Reg. no. 51 929), batch no. COD-001416, purity: 100.0% (tolerance ± 1.0%).
Test soil:	Biologically active agricultural soil: loamy sand soil; pH 6.6, 1.35% C_{org} and 36.19% WHC.
Test design:	Determination of carbon-transformation (O_2-consumption) in soil after addition of glucose resulting in a soil concentration of 0.4%. Three treatment groups were set up (one untreated control group and two concentrations of the test item) with three replicates per treatment. The O_2-consumption was measured using a “BSB-digi” respirometer system over a period of 12 hours at different sampling intervals. Sampling scheme: 0, 7, 14 and 28 days after treatment, sub-samples were withdrawn from the bulk batches and subjected to measurement.
Endpoint:	Concentration producing maximum 25% deviation in O_2-consumption compared to the control.

Test concentrations: Control, 1.92 mg bentazone/kg dry soil and 19.20 mg bentazone/kg dry soil.

Reference item: Dinoterb (purity: 99.9%). The reference item was tested in a separate study at rates of 6.8, 16.0 and 27.0 mg Dinoterb/kg dry soil.

Test conditions: Soil moisture: approx. 45% of its water holding capacity corresponding to a measured water content of 16.88% - 17.74%, pH 6.2 - 6.4. Soil samples were incubated at 18.2 °C - 21.6 °C while stored in steel vessels in the dark.

Statistics: Descriptive statistics.

II. RESULTS AND DISCUSSION

No significant influences of bentazone on the carbon transformation were observed after 28 days, only slight deviations from the control of -2.2% and -23.2% were measured at the concentrations of 1.92 and 19.20 mg/kg dry soil. The results are summarized in Table 8.10/4.

Table 8.10/4 Effects of bentazone on soil micro-organisms (carbon transformation) on days 7, 14 and 28of incubation

Soil (days)	Control	1.92 mg bentazone/kg dry soil		19.20 mg bentazone/kg dry soil	
	O_2 Consumption [mg/kg dry soil/h]	O_2 Consumption [mg/kg dry soil/h]	% Deviation from control [1)]	O_2 Consumption [mg/kg dry soil/h]	% Deviation from control [1)]
Loamy sand soil (7 d)	8.93	8.81	-1.3	7.77	-13.0
Loamy sand soil (14 d)	8.71	8.59	-1.4	7.41	-14.9
Loamy sand soil (28 d)	9.74	9.53	-2.2	7.48	-23.2

[1)] Based on O_2 consumption - = inhibition; + = stimulation

In a separate study the reference item Dinoterb caused effects on the C-transformation of -30.5%, -34.5% and -28.8% at test concentrations of 6.8, 16.0 and 27.0 mg Dinoterb/kg dry soil, respectively.

III. CONCLUSION

Based on the results of this study, bentazone caused no short-term and no long-term effects on the carbon transformation (measured as oxygen consumption) in a loamy sand field soil tested up to a concentration of 19.20 mg bentazone/kg dry soil.

Report:	II A 8.10.2/2 Schulz L. 2004(d) Effects of Reg.No. 79520 (BH 351-N-Me, metabolite of BAS 351 H, Bentazon) on the activity of soil microflora (Carbon transformation test) BASF DocID 2004/1014994
Guidelines:	OECD 217 (2000)
Deviations:	None
GLP:	Yes (laboratory certified by Saechsisches Staatsministerium fuer Umwelt und Landwirtschaft, Dresden, Germany)

Executive Summary

The effect of N-methyl-bentazone (M351H009) on soil respiration was investigated in a sandy loam soil with 0.4 and 2.0 mg N-methyl-bentazone/kg dry soil, corresponding to 0.275 kg test item/ha and 1.375 kg test item/ha, respectively. Three samples of each treatment were removed for analysis of carbon content 7, 14 and 28 days after application.

After 28 days, there were no significant effects on soil respiration at any application rate.

N-methyl-bentazone (M351H009) caused no adverse effects (deviation from control < 25%) on the soil C-transformation (measured as oxygen consumption) at the end of the 28 day incubation period. The study was performed in sandy loam soil at concentrations up to 2.0 mg N-methyl-bentazone/kg dry soil, corresponding to 1.375 kg test item/ha.

I. MATERIAL AND METHODS

Test item:	N-methyl-bentazone (M351H009), batch No. 2235-09, purity: 99.8%.
Test species:	Biologically active agricultural soil: sandy loam soil; pH 6.9, 1.49% C_{org}, 44.5% WHC, 11.3 cmol/kg dry soil CEC.
Test design:	Determination of carbon-transformation (O_2-consumption) in soil after the addition of glucose. Comparison of test item treated soil with a non-treated soil. 3 replicates per treatment and concentration were set up. A respirometer system was used to measure the O_2-consumption over a period of maximum 24 hours at different sampling intervals. Sampling scheme: 0, 7, 14 and 28 days after treatment, sub-samples were withdrawn from the bulk batches and subjected to measurement.
Endpoints:	Effects on O_2 consumption after 28 days of exposure.

Test concentrations: Control, 0.4 and 2.0 mg N-methyl-bentazone/kg dry soil, corresponding to 0.275 kg test item/ha and 1.375 kg test item/ha, respectively. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g dry soil/cm^3.

Reference item: Dinosep acetate, 8.67 mg/kg dry soil; effects have been evaluated in a separate study.

Test conditions: Soil moisture: 45% of its maximum water holding capacity. Soil samples were incubated at 20 °C ± 2 °C while stored in new plastic vessels.

Statistics: Mean values per treatment, standard deviations and coefficients of variation.

II. RESULTS AND DISCUSSION

No adverse effects of N-methyl-bentazone on carbon transformation in soil were observed at both application rates after 28 days. Only slight deviations from the control of -2.2% and -2.4% were measured in the concentrations in the sandy loam soil at day 28. The results are summarized in Table 8.10/5.

Table 8.10/5 Effects of N-methyl-bentazone on short term soil respiration at day 28 of incubation.

Soil (days)	Control	0.4 mg test item/kg dry soil		2.0 mg test item/kg dry soil	
	O_2 consumption [mg/kg dry soil/h]	O_2 consumption [mg/kg dry soil/h]	% Deviation from control [1)]	O_2 consumption [mg/kg dry soil/h]	% Deviation from control [1)]
Sandy loam soil (7 d)	12.47	12.46	-0.1	12.09	-3.1
Sandy loam soil (14 d)	11.71	11.56	-2.2	11.32	-3.4
Sandy loam soil (28 d)	11.28	16.4	-2.2	17.3	-2.4

[1)] Based on O_2 consumption; - = inhibition; + = stimulation

The reference item produced the expected level of effect on carbon transformation (25.4% inhibition after 28 days).

III. CONCLUSION

N-methyl-bentazone (M351H009) caused no adverse effects (deviation from control < 25%) on the soil C-transformation (measured as oxygen consumption) at the end of the 28 day incubation period. The study was performed in sandy loam soil at concentrations up to 2.0 mg N-methyl-bentazone/kg dry soil, corresponding to 1.375 kg test item/ha.

8.10.3 Rates of recovery following treatment

Not applicable, because bentazone is not used for soil sterilization. In addition, no adverse effects were observed neither in the nitrogen transformation nor in the carbon transformation studies.

Summary of results

Table 8.10/6 Summary of effects of bentazone, bentazone-Na and its metabolite N-methyl-bentazone on soil microbial activities

Test system/ Soil type	Application rate [mg/kg dry soil]	Result [%] deviation from control [1)]	Reference
Bentazone			
N-transformation			
Silty sand (28 d)	1.92	+19.8	Schulz, 2010/1144223
Silty sand (56 d)	5.76	+13.8	
C-transformation			
Loamy sand (28 d)	1.92	-2.2	Schulz, 2010/1144219
	19.20	-23.2	
Bentazone-Na			
N-transformation			
Silty sand (42 d)	2.09	+15.4	Schulz, 2011/1057030
	6.27	+24.6	
N-methyl-bentazone			
N-transformation			
Sandy loam (28 d)	0.4	-1.3	Schulz, 2004/1014995
	2.0	-0.7	
C-transformation			
Sandy loam (28 d)	0.4	-2.2	Schulz, 2004/1014994
	2.0	-2.4	

[1)] - = inhibition; + = stimulation

8.11 Effects on marine and estuarine organisms

8.11.1 Marine or estuarine organisms acute toxicity LC50/EC50

The following studies with salt water species were not submitted in the last EC review of bentazone since saltwater organisms were not required and none of the endpoints is lower than the standard studies. However, due to data requirements in US, these studies were performed and are available. Since in future saltwater organisms, especially *Mysidopsis bahia,* will be required for special mode of actions, the full salt water data package is presented here.

A study summary with the marine diatom *Skeletonema costatum* is provided in the updated chapter 8.04 (Dez. 2013) together with the other algae studies.

Reference number:	II A 8.11.1/1
Report:	[redacted] 1991(a) Bentazon: A 96-hour flow-through acute toxicity test with the sheepshead minnow (Cyprinodon variegatus) [redacted] unpublished BASF DocID 1991/5191
Guidelines:	EPA 72-3; EPA-Guideline - Pesticide Assessment Guidelines. Subdiv. E Hazard Evaluation Wildlife and Aquatic Organisms
GLP:	Yes (laboratory certified by United States Environmental Protection Agency)

Executive Summary

In a flow-through acute toxicity laboratory study, sheepshead minnow were exposed to bentazone at a single concentration of 120 mg a.s./L (nominal) in groups of 10 animals in polyethylene aquaria containing 6.5 L water with 3 replicates per concentration. Fish were observed for survival and symptoms of toxicity 4, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations. After 96 hours of exposure, no mortality and toxic effects were observed in the control and at the tested concentration of 136 mg a.s./L.

In a flow-through acute toxicity study with sheepshead minnow (*Cyprinodon variegatus*) the LC_{50} (96 h) for bentazone was > 136 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be ≥ 136 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

Test item:	Bentazone (BAS 351 H, Reg. No. 51 929), lot no. 68-901102, purity: 53.0%.
Test species:	Sheepshead minnow (*Cyprinodon variegatus*), juvenile, mean body length 2.6 cm (2.3 - 3.0 cm); mean body weight 0.58 g (0.31 - 0.96 g); source: in-house cultures.
Test design:	Flow-through system (96 hours); 10 fish per aquarium (loading: 0.12 g fish/L passing through test chambers in 24 hours, instantaneous loading: 0.90 g fish/L present in test chambers), 3 replicates per concentration; assessment of mortality and symptoms of toxicity 4, 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50}, NOEC, mortality and sub-lethal effects.
Test concentrations:	Control (dilution water), 120 mg bentazone/L (nominal); corresponding to 136 mg a.s./L (mean measured).
Test conditions:	8 L polyethylene tanks, test volume 6.5 L, filtered seawater, salinity: 25‰; temperature: 21.8 °C - 22.2 °C; pH 8.1 - 8.4; oxygen content: 5.4 mg/L - 6.4 mg/L; photoperiod 16 h light : 8 h dark; light intensity: 969 lux; no feeding.
Analytics:	Analytical verification of test item concentrations was conducted using a GC-method with thermionic specific detection.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted at test initiation and after 24, 48, 72 and 96 hours. Measured concentrations of bentazone ranged from 102% to 127% of nominal. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure no mortality and toxic effects were observed in the control and at the tested concentration of 136.0 mg a.s./L (mean measured). The results are summarized in Table 8.11/7.

Table 8.11/7 Acute toxicity (96 h) of bentazone to sheepshead minnow (*Cyprinodon variegatus*)

Concentration [mg a.s./L] nominal	**Control**	**120.0**
Concentration [mg a.s./L] mean measured	**--**	**136.0**
Mortality [%]	0	0
Symptoms	none	none
	Endpoints [mg bentazone/L] (mean measured)	
LC_{50} (96 h)	> 136.0	
NOEC (96 h)	≥ 136.0	

III. CONCLUSION

In a flow-through acute toxicity study with sheepshead minnow (*Cyprinodon variegatus*), the LC_{50} (96 h) for bentazone was > 136.0 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be ≥ 136.0 mg a.s./L (mean measured).

Executive Summary

I. MATERIAL AND METHODS

II. RESULTS AND DISCUSSION

III. CONCLUSION

Report:	II A 8.11.1/2 Graves W.C., Smith G.J. 1991(a) Bentazon: A 96-hour flow-through acute toxicity test with the saltwater mysid (Mysidopsis bahia) BASF DocID 1991/5192
Guidelines:	EPA-Guideline - Pesticide Assessment Guidelines. Subdiv. E Hazard Evaluation Wildlife and Aquatic Organisms; EPA 72-3 **Deviations:** none
GLP:	Yes (laboratory certified by United States Environmental Protection Agency)

Executive Summary

In a flow-through acute toxicity laboratory study, saltwater mysids were exposed to bentazone at a single concentration of 120.0 mg a.s./L (nominal) in groups of 10 animals in 500 mL glass beakers suspended in 8 L polyethylene test chambers containing 6.5 L test solution with 3 replicates per treatment. Saltwater mysids were observed for survival and symptoms of toxicity 4, 24, 48, 72 and 96 hours after start of exposure.

The biological results are based on mean measured concentrations. After 96 hours of exposure one mysid was absent in the control and assumed to be dead. In the 132.5 mg a.s./L treatment group one single mysid died; however, this was not considered to be a treatment related effect. No sub-lethal effects were observed in the control and in the test item treatment group after 96 hours of exposure.

In a flow-through acute toxicity study with saltwater mysids (*Mysidopsis bahia*) the LC_{50} (96 h) for bentazone was > 132.5 mg a.s./L based on mean measured concentrations. The NOEC (96 h) determined to be ≥ 132.5 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

Test item:	Bentazone (BAS 351 H, Reg. No. 51 929), lot no. 68-901102, purity: 53.0%.
Test species:	Saltwater mysid (*Mysidopsis bahia*), juveniles, age: less than 24 hours old; source: in-house cultures.
Test design:	Flow-through system (96 hours); 10 mysids per test chamber, 3 replicates per treatment; assessment of mortality and symptoms of toxicity 4, 24, 48, 72 and 96 hours after start of exposure.
Endpoints:	LC_{50} (96 h), NOEC, mortality and sub-lethal effects.
Test concentrations:	Control (dilution water), 120.0 mg bentazone/L (nominal); corresponding to 132.5 mg a.s./L (mean measured).
Test conditions:	500 mL glass beakers suspended in 8 L polyethylene test chambers filled with 6.5 L test solution; filtered seawater, salinity: 25‰; temperature: 24.7 °C - 25.1 °C; pH 8.1 - 8.4; oxygen content: 5.8 mg/L - 6.2 mg/L; photoperiod 16 h light : 8 h dark; light intensity: 969 lux at test initiation; juvenile mysids were fed daily with live brine shrimps (*Artemia* sp.).
Analytics:	Analytical verification of test item concentrations was conducted using a GC-method with thermionic specific detection.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted at test initiation and after 24, 48, 72 and 96 hours. Mean measured concentrations for bentazone ranged from 101% to 120% of nominal. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure one mysid was absent in the control and assumed to be dead. In the 132.5 mg a.s./L treatment group one single mysid died; however, this was not considered to be a treatment related effect. No sub-lethal effects were observed in the control and in the test item treatment group after 96 hours of exposure. The results are summarized in Table 8.11/8.

Table 8.11/8 Acute toxicity (96 h) of bentazone to saltwater mysids (*Mysidopsis bahia*)

Concentration [mg a.s./L] nominal	**Control**	**120.0**
Concentration [mg a.s./L] mean measured	**--**	**132.5**
Mortality [%]	3.3	3.3
Symptoms	none	none
	Endpoints [mg bentazone/L] (mean measured)	
LC_{50} (96 h)	> 132.5	
NOEC (96 h)	≥ 132.5	

III. CONCLUSION

In a flow-through acute toxicity study with saltwater mysids (*Mysidopsis bahia*) the LC_{50} (96 h) for bentazone was > 132.5 mg a.s./L based on mean measured concentrations. The NOEC (96 h) was determined to be ≥ 132.5 mg a.s./L (mean measured).

Reference number: II A 8.11.1/3
Report: Graves W.C., Smith G.J. 1992(a)
Bentazon: A 96-hour shell deposition test with the eastern oyster (Crassostrea virginica)
Wildlife International Ltd.; Easton MD; United States of America
unpublished
BASF DocID 1992/5017
Guidelines: EPA 72-3; EPA-Guideline - Pesticide Assessment Guidelines. Subdiv. E Hazard Evaluation Wildlife and Aquatic Organisms
GLP: Yes
(laboratory certified by United States Environmental Protection Agency)

Executive Summary

A study was conducted to determine the effects of bentazone on the shell deposition of eastern oysters during a 96-hour exposure period under flow-through test conditions. The eastern oysters were exposed to 15.6, 25.9, 43.2, 72.0 and 120.0 mg a.s./L (nominal) in groups of 20 oysters in polyethylene aquaria containing 12.6 L seawater per concentration. Eastern oysters were observed twice daily for survival and symptoms of toxicity. Measurements of shell deposition were made after 96 hours and used to calculate the EC_{50} and the NOEC value.

The biological results are based on mean measured concentrations. After 96 hours of exposure, no mortalities and no sub-lethal effects were observed in the control and in any test item treatment group. Oyster shell growth in the control averaged 4.1 mm over the 96-hour exposure period. Mean shell growth in the test item treatment groups ranged from 2.53 to 3.18 mm, resulting in shell growth inhibition between 22.4% and 38.3% compared to the control.

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the EC_{50} (96h) for bentazone was > 109 mg a.s./L based on mean measured concentrations. The NOEC was determined to be < 10 mg a.s./L (mean measured).

I. MATERIAL AND METHODS

Test item:	Bentazone (BAS 351 H, Reg. No. 51 929), lot no. 68-901102, purity: 53.0%.
Test species:	Eastern oysters (*Crassostrea virginica*), juveniles with average length of 41 mm (range: 35 - 47 mm); source: P. Cummins Oyster Company, Pasadena (MD), USA.
Test design:	Flow-through system (96 hours); 20 oysters per aquarium and per concentration; assessment of mortality and symptoms of toxicity twice daily and measurements of shell deposition 96 hours after start of exposure.
Endpoints:	EC_{50} (96 h) and NOEC for shell growth inhibition; mortality and symptoms of toxicity.
Test concentrations:	Control (dilution water), 15.6, 25.9, 43.2, 72.0 and 120 mg bentazone/L (nominal); corresponding to 10.0, 19.0, 29.0, 61.0 and 109.0 mg a.s./L (mean measured).
Test conditions:	56 L polyethylene aquaria, test volume 12.6 L; unfiltered seawater, salinity: 24‰ - 26‰; temperature: 21.6 °C - 22.0 °C; pH 7.9 - 8.1; oxygen content: 6.4 mg/L - 7.3 mg/L; photoperiod 16 h light : 8 h dark; light intensity: 409 lux; supplementary diet for oysters: suspension of marine microalgae.
Analytics:	Analytical verification of test item concentrations was conducted using a GC-method with thermionic specific detection.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

Analytical measurements: Analytical verification of test item concentration was conducted in each concentration at test initiation and after 48 and 96 hours. Mean measured concentrations of bentazone ranged from 64% to 91% of nominal concentrations. The following biological results are based on mean measured concentrations.

Biological results: After 96 hours of exposure, no mortalities and no sub-lethal effects were observed in the control and in any test item treatment group. Oyster shell growth in the control averaged 4.1 mm over the 96-hour exposure period. Mean shell growth in the test item treatment groups ranged from 2.53 to 3.18 mm, resulting in shell growth inhibition between 22.4% and 38.3% compared to the control. The results are summarized in Table 8.11/9.

Table 8.11/9 Acute toxicity (96 h) of bentazone to eastern oysters (*Crassostrea virginica*)

Concentration [mg a.s./L] nominal	**Control**	**15.6**	**25.9**	**43.2**	**72.0**	**120.0**
Concentration [mg a.s./L] mean measured	**--**	**10.0**	**19.0**	**29.0**	**61.0**	**109.0**
Shell growth inhibition after 96h [%] [1)]	--	30.7	22.4	31.2	28.8	38.3
Mortality after 96h [%]	0	0	0	0	0	0
Symptoms	none	none	none	none	none	none
Endpoints [mg bentazone/L] (mean measured)						
EC_{50} (96 h)	> 109					
NOEC (96 h) [2)]	< 10					

[1)] Percentage of mean growth observed in control.
[2)] Based on visual inspection of the data.

III. CONCLUSION

In a flow-through acute toxicity study with eastern oysters (*Crassostrea virginica*), the EC_{50} (96h) for bentazone was > 109 mg a.s./L based on mean measured concentrations. The NOEC was determined to be < 10 mg a.s./L (mean measured).

8.11.2 Marine/Estuarine fish - salinity challenge

Studies on marine or estuarine species are not required according to the relevant EU documents; however, in the context of registration for the USA such studies have been performed and the results are listed below [see Table 8.11/10] and in Document M-III, chapter 10.2.

The contamination of estuarine and marine environments is considered to be minimal compared to freshwater habitats adjacent to agricultural land according to the use pattern, the potential route of contamination and the dissipation of the active ingredients. Thus, the risk to those habitats is covered by the risk assessment for freshwater ecosystems. Therefore, the saltwater species have not been further considered.

Summary of results

Results of toxicity studies with bentazone on marine organisms are summarized in Table 8.11/10.

Table 8.11/10 Summary of effects of bentazone on marine and estuarine organisms

Test species	Test system	Test concentration	Result [mg/L]		Reference
			LC_{50}/EC_{50}	NOEC	
Bentazone					
Cyprinodon variegatus	Flow-through 96 h	mean measured	> 136.0	≥ 136.0	[redacted] 1991/5191
Mysidopsis bahia	Flow-through 96 h	mean measured	> 132.5	≥ 132.5	Graves & Smith 1991/5192
Crassostrea virginica	Flow-through 96 h	mean measured	> 109.0	< 10.0	Graves & Smith 1992/5017

A comprehensive risk assessment for aquatic organisms can be found in Document M-III of BAS 351 32 H chapter 10.2.

8.12 Effects on terrestrial vascular plants

Studies on non-target plants have been performed with BAS 351 32 H and are reported in Document M-III, Chapter 10.8.

8.13 Effects on terr. vertebrates other than birds / wild mammal toxicity

A comprehensive risk assessment can be found in Document M III, Chapter 10.3.

The information as to the toxicity of the active substance bentazone and the formulated product BAS 351 32 H, which is required for the risk assessment on wild mammals, is provided in Document M-II, Chapter 5 and Document M-III, Chapter 7, respectively. No additional information is deemed necessary for the risk assessment.

8.14 Effects on other non-target organisms believed to be at risk

8.14.1 Summary of preliminary data: biological activity & dose range finding

Observations on adverse effects of bentazone on other non-target organisms did not indicate particular risks during the screening and developmental phase.

Studies on non-target plants and non-target arthropods have been performed with BAS 351 32 H or BAS 351 45 H and are reported in the respective Document M-II, Chapter 8 and Document M-III, Chapter 10.

8.14.2 Assessment of relevance to potential impact on non-target species

The assessment on non-target species is presented in Document M-III, Chapter 10.

8.15 Effects on biological methods for sewage treatment

Report:	II A 8.15/1 Bachner H. 2004(a) BAS 351 H (Bentazone) - Determination of the inhibition of oxygen consumption by activated sludge in the activated sludge respiration inhibition test BASF DocID 2004/1025743
Guidelines:	OECD 209; EEC 88/302
Deviations:	none
GLP:	Yes (laboratory certified by Landesanstalt fuer Pflanzenbau und Pflanzenschutz Rheinland-Pfalz, Mainz)

Executive Summary

The effect of bentazone on the respiration rate of activated sludge collected from a laboratory wastewater plant was determined. Bentazone was tested at a concentration of 1000 mg a.s./L. Oxygen consumption rate of aerobic micro-organisms was assessed after a contact period of 180 min under aeration.

The biological results are based on the nominal concentration. No significant inhibition of respiration was measured at 1000 mg a.s./L (nominal).

The EC_{20} and EC_{50} values of bentazone in the activated sludge respiration inhibition test are both > 1000 mg a.s./L, respectively. Disturbances in the bio-degradation process of activated sludge are not to be expected if the test item is correctly introduced into adapted wastewater treatment plants at low concentrations.

I. MATERIAL AND METHODS

Test item:	Bentazone (Reg. no. 51 929), batch no. N 187, purity: 96.9%.
Test species:	Activated sludge from laboratory wastewater plant treating municipal sewage.
Test design:	Assessment of the inhibitory effect of the test item on the oxygen consumption rate of aerobic micro-organisms (activated sludge) after short-term exposure of 180 min; the inoculum was aerated during the contact period.
Test concentrations:	Control, 1000 mg bentazone/L.
Reference item:	3.5-dichlorophenol. The reference item was applied at 1, 10 and 100 mg/L.
Test conditions:	Temperature: 20 °C ± 2 °C; pH-value was adjusted to 7.5 ± 0.5; 250 mL glass vessels, 250 mL of test mixture per vessel.
Analytics:	Not applicable.
Statistics:	Descriptive statistics.

II. RESULTS AND DISCUSSION

No significant inhibition of respiration was measured at 1000 mg bentazone/L (nominal).

III. CONCLUSION

The EC_{20} and EC_{50} values of bentazone in the activated sludge respiration inhibition test are both > 1000 mg a.s./L, respectively. Disturbances in the bio-degradation process of activated sludge are not to be expected if the test item is correctly introduced into adapted wastewater treatment plants at low concentrations.

8.16 Other/special studies

No other or special studies than those detailed under Document M-II, Chapters 8.1 to 8.15 were performed or are deemed to be necessary.

8.16.1 Other/special studies - laboratory studies

No other/special laboratory studies than those detailed under Document M-II, Chapters 8.1 to 8.15 were performed or are deemed to be necessary.

8.16.2 Other/special studies - field studies

No special ecotoxicological field studies with the active ingredient bentazone are deemed to be necessary.

8.17 Summary and evaluation of points IIA 7 and IIA 8.1 to 8.16

Environmental Fate and Behaviour

Soil behaviour
The major route of bentazone degradation in soil is the formation of bound residues with tight incorporation into all fractions of the organic soil matrix (up to 64% of the applied radioactivity (AR) distributed between fulvic acids, humic acids and humins after 120 days). First metabolization step is assumed to be hydroxylation and the phenyl-moiety. The resulting 6- or 8-OH-bentazones are then quickly incorporated into the humic substances. Mineralization reached 8% within 120 days. DT_{50} values under laboratory conditions at 20°C were in the range of 17 - 49 days, DT_{90} values 56 - 163 days. Under field conditions, bentazone degraded with half-lives of 3.9 - 26.4 days.

The degradation is negligible under anaerobic conditions ($DT_{50anaerobic}$ > 1000 days). A soil photolysis study showed a faster degradation under irradiated conditions compared to dark conditions ($DT_{50irrad.}$ 12.8 days (continuous. irrad.), $DT50_{dark\ control}$ 42.1 days), however, no new metabolites appeared. It can be concluded that the route of degradation is still the same under sunlight, only the formation rate of bound residues seems to be enhanced.

N-methyl-bentazone was the only metabolite reaching barely 5% of the initially applied radioactivity (AR) during aerobic soil incubation. No other peak exceeded 4% AR in any of the new bentazone studies. The degradation rate of N-methyl-bentazone was determined in a separate metabolite study and was calculated to be 38 - 153 days (SFO kinetics).

Aquatic behaviour
Bentazone is hydrolytically stable at all tested pH values. Photolysis may be an influencing factor in the aquatic environment, since bentazone was degraded fast at all pHs to about 3.6-16.5% AR during 15 days continuous irradiation with half-lives of 3.3 days (pH 5), 5.4 days (pH 7) and 3.9 days (pH 9). Two major photodegradates were identified with max. amounts of 30% ("Peak B") and 25% AR ("Peak C"). Both metabolites were tested for aquatic toxicity and proved to have no harmful effects.

In an aerobic aquatic metabolism study with two water/sediment systems ("Krempe" and "Ohlau"), a high portion of bentazone remained in the water phase (53 - 61% AR after 100 days). The maximum observed occurrence of bentazone in the sediment reached a maximum of 12.2% AR at day 30 after treatment in system "Krempe". It slowly declined in sediment to 9.6% at 100 days.

N-methyl-bentazone was the only major metabolite found at a maximum of 12.5% AR after 30 days in the water phase. It decreased again to about 2% after 100 days. N-methyl-bentazone is not expected to occur in significant amounts in sediments. Mineralization was negligible with 2.6% AR after 100 days. Formation of non-extractable residues was moderate reaching 13.4 - 15.6% AR at the end of incubation.

A new kinetic evaluation of the water/sediment study revealed half-lives in water of 156 - 204 days, in sediment 171 - 179 days and in the whole system 207 - 283 days.

Ecotoxicological studies on the active substance

The relevant endpoints of all studies mentioned in Annex II under point 8.1 to 8.16 are summarized below. The evaluation of risk is detailed in Doc M-III chapter 10.1 to 10.11 of the formulation BAS 351 32 H.

Effects on terrestrial vertebrates (OECD data point numbers Annex IIA, 8.1)

For the assessment of effects of bentazone on **birds**, tests with mallards (*Anas platyrhynchos*) and bobwhite quails (*Colinus virginianus*) were conducted. The studies performed cover a broad range of biological endpoints, such as mortality, signs of toxicity, feed consumption, body weight, post-mortem examinations and various reproduction parameters.

The acute oral toxicity test with the bobwhite quail resulted in a low acute toxicity of bentazone to birds. The LD_{50} value was about 1140 mg a.s./kg b.w.

The short-term dietary toxicity studies showed that bentazone has a low toxicity to hatchlings of quails and mallard ducks. The LC_{50} values were > 5000 mg a.s./kg b.w. for both species.

In a 22-week avian reproduction test with mallard ducks (*Anas platyrhynchus*), no substance-related adverse effects on mortality, health, palatability, food consumption and body weight of the parental generation could be detected up to 800 mg a.s./kg diet, the highest dose in the test. The NOEC for reproduction of bentazone was determined to be 800 mg a.s./kg diet corresponding to a NOEL of 129 mg a.s./kg b.w./day.

In a 22-week avian reproduction test with bobwhite quails (*Colinus virginianus*), no substance-related adverse effects on mortality, health, palatability, food consumption and body weight of the parental generation could be detected up to 800 mg a.s./kg diet, the highest dose in the test. The NOEC for reproduction of bentazone was determined to be 800 mg a.s./kg diet corresponding to a NOEL of 75.44 mg a.s./kg b.w./d.

Acute toxicity to birds	**Bentazone:** *C. virginianus*:	 LD_{50}: about 1140 mg a.s./kg b.w.
Dietary toxicity to birds	**Bentazone:** *C. virginianus* *A. platyrhynchos*	 LC_{50} > 5000 mg a.s./kg b.w./day *) LC_{50} > 5000 mg a.s./kg b.w./day *)
Reproductive toxicity to birds	**Bentazone:** *A. platyrhynchos* *C. virginianus*	 NOEC ≥ 800 a.s./kg diet NOEL = 129 mg a.s./kg b.w./day *) NOEC ≥ 800 mg a.s./kg diet NOEL = 75.44 mg a.s./kg b.w./day *)
*) Daily Dose (mg a.s./kg bw/day) calculated based mean feed consumption and mean body weight data		

For the assessment of effects of bentazone on **mammals**, toxicity tests on rats were conducted whose results are summarized in the Documents M-II 5.

Effects on aquatic species (OECD data point numbers Annex IIA, 8.2 - 8.6; 8.11)

The effects of the active substance bentazone and its metabolites on aquatic organisms are reported in detail in Document M-II 8.2 to M-II 8.6 and M-II 8.11; they are summarized in the table below.

For bentazone, the studies were performed with the free acid of the active substance, with DEA- or Na-salts or with a formulation containing the Na-salt of bentazone without formulants which might influence the toxicity to aquatic organisms. The equivalency of bentazone acid and bentazone salt regarding ecotoxicological effects could be demonstrated with studies on the most sensitive species for bentazone, *Lemna gibba*.

The acute tests performed with bentazone showed low toxicity to fish and daphnids with LC_{50} values of > 100 mg a.s./L. In toxicity tests with different alga species the E_bC_{50} ranged from 10.1 to 71 mg a.s./L and the E_rC_{50} obtained in the study on *P. subcapitata* was 33.3 mg a.s./L. The toxicity to higher aquatic plants as represented by *Lemna gibba* was also moderate with E_rC_{50} and E_yC_{50} ranging from 22.2 to 25.0 mg a.s./L and from 5.35 to 9.7 mg a.s./L, respectively.

Chronic toxicity of bentazone to aquatic organisms was tested in a 21-day study on daphnids, in a 28-day study on rainbow trout and in a 35-day fish early life stage test on fathead minnow. The lowest chronic NOEC was ≥ 10 mg a.s./L obtained for fish in the ELS study whereas daphnids were less sensitive with a NOEC of 120 mg a.s./L.

The metabolite N-methyl-bentazone (= M351H009) showed higher toxic potential to fish (LC_{50} = 8.56 mg/L) and *Daphnia* (EC_{50} = 26.5 mg/L) compared to the parent compound. Therefore, additional chronic tests on fish and daphnids were conducted resulting in NOEC values of 0.23 mg/L and 2.0 mg/L, respectively. The results obtained for the metabolite N-methyl-bentazone and aquatic primary producers, the most sensitive group to the active substance bentazone, did not show increased toxicity but the results were in a similar range than those obtained in the studies on algae and *Lemna gibba* with bentazone.
The metabolites Peak B (= M351H023) and Peak C (=M351H024) showed identical toxic potential to *Daphnia* and much less toxic potential to the aquatic plant *L. gibba* compared to the parent compound with EC_{50} values of > 100 mg/L in all studies for both metabolites. Since the metabolites Peak B and Peak C showed no toxicity to *Lemna* and *Daphnia*, and *Lemna* represents the most sensitive species for the a.s., as well as for animal welfare reasons, no additional fish study was conducted with the metabolites Peak B and Peak C. Furthermore, no chronic studies were performed with these metabolites.

Since bentazone is not toxic to daphnia (NOEC (21 d) = 120 mg/L) and it does not adsorb to a significant extent to the sediment, no test on sediment-dwelling organisms was performed.

Group	Test item	Time-scale	Endpoint	Toxicity (mg/L)
Laboratory tests with bentazone and metabolites				
Fish species				
O. mykiss, static	bentazone	96 h	LC_{50} / NOEC	> 100 / 50
P. promelas, static	bentazone [1)]	96 h	LC_{50} / NOEC	> 120 / ≥ 120
L. macrochirus, static	bentazone	96 h	LC_{50} / NOEC	> 100 / 100
C. carpio, static	bentazone [1)]	96 h	LC_{50} / NOEC	> 1000 / 560
C. variegatus, static [+]	bentazone	96 h	LC_{50} / NOEC	> 136 / ≥ 136
O. mykiss, static	N-methyl-bentazone (= M351H009)	96 h	LC_{50} / NOEC	8.56 / 3.9
O. mykiss, flow-through	bentazone [2)]	28 d	NOEC	48
P. promelas, ELS, flow-through	bentazone [1)]	35 d	NOEC	≥ 10
O. mykiss, flow-through	N-methyl-bentazone	28 d	NOEC	0.23
Invertebrate species				
D. magna, static	bentazone	48 h	EC_{50} / NOEC	125 / 62.5
D. magna, static	bentazone	48 h	EC_{50} / NOEC	> 100 / ≥ 100
M. bahia, flow-through *	bentazone	96 h	LC_{50} / NOEC	> 132.5 / ≥ 132.5
C. virginica, flow-through *	bentazone	96 h	EC_{50} / NOEC	> 109 / < 10
D. magna, static	N-methyl-bentazone	48 h	EC_{50} / NOEC	26.5 / 15.3
D. magna, static	Peak B (= M351H023)	48 h	EC_{50} / NOEC	> 100 / ≥ 100
D. magna, static	Peak C (= M351H024)	48 h	EC_{50} / NOEC	> 100 / ≥ 100
D. magna, semi-static	bentazone [2)]	21 d	NOEC	120
D. magna, semi-static	N-methyl-bentazone	21 d	NOEC	2.0
Algal species				
P. subcapitata, static	bentazone	72 h	E_rC_{50} / E_rC_{10} E_bC_{50} / E_bC_{10}	33.3 / 9.89 16.8 / 7.90
A. bibraianus, static	bentazone	72 h	E_bC_{50} / E_bC_{10}	62 [#] / 1.5 [#]
A. bibraianus, static	bentazone [3)]	72 h	E_bC_{50} / E_bC_{10}	71 [#] / 5.0 [#]
A. flos-aquae, static	bentazone	120 h	E_bC_{50} / E_bC_{25} NOEC	10.1 / 6.9 6.3
N. pelliculosa, static	bentazone	120	E_bC_{50} / E_bC_{25} NOEC	> 14.73 / > 14.73 14.73
S. costatum, static *	bentazone	120 h	E_bC_{50} / E_bC_{25} NOEC	10.1 / 6.31 3.72
P. subcapitata, static	N-methyl-bentazone	72 h	E_rC_{50} / E_rC_{10} E_yC_{50} / E_yC_{10}	37.7 / 9.8 15.8 / 5.2
Aquatic plant species				
L. gibba, static	bentazone	7 d	E_rC_{50} / E_rC_{10} * E_yC_{50} / E_yC_{10} *	25.0 / 3.3 9.2 / 3.8
L. gibba, static	bentazone [1)]	7 d	E_rC_{50} / E_rC_{10} * E_yC_{50} / E_yC_{10} *	22.2 / 3.9 9.7 / 3.4
L. gibba, static	bentazone [3)]	14 d	E_bC_{50} / E_bC_{25} NOEC *	5.35 / 3.57 3.06
L. gibba, static	N-methyl-bentazone	7 d	E_rC_{50} / E_rC_{10} * E_bC_{50} / E_bC_{10} *	35.84 / 13.93 21.32 / 9.0
L. gibba, static	Peak B	7 d	E_rC_{50} / E_rC_{10} * E_yC_{50} / E_yC_{10} *	> 100 / > 100 > 100 / > 100
L. gibba, static	Peak C	7 d	E_rC_{50} / E_rC_{10} * E_yC_{50} / E_yC_{10} *	> 100 / > 100 > 100 / > 100

* Marine species # graphically determined + Results based on frond number
1) Study was conducted with the Na-salt of bentazone.
2) Study was conducted with the solo formulation BAS 351 32 H (Basagran), containing 48% bentazone.
3) Study was conducted with the DEA-salt of bentazone; the endpoint presented was calculated on the basis of free bentazone.

Bioconcentration

The log P_{ow} of the active substance bentazone was determined to be < 3. The accumulation potential of bentazone in aquatic non-target organisms is hence considered to be low. Additionally, a bioaccumulation study in bluegill sunfish has been performed with bentazone. The bioconcentration factor in whole fish was determined to be 1.4 at steady state and the depuration half life was 13.9 days (whole fish). The low bioconcentration factors and the rapid elimination of radioactive residues demonstrate a low bioconcentration potential for bentazone and thus, it is concluded that there is no risk of bioaccumulation in food chains.

Bioconcentration

Bioconcentration factor (BCF)	BCF of 1.4 (whole fish)
Risk assessment trigger (practical conditions of use) for the bioconcentration factor *	100
Clearance time (CT_{50})	DT_{50} (depuration half-life) = 13.9 days (whole fish)
Level of residues (%) in organisms after the 14 day depuration phase	Not relevant

* In the EU a risk assessment must be carried out relevant to practical conditions of use where the BCF value reported is greater than this value

Effects on honeybees (OECD data point numbers Annex IIA, 8.7)

An acute oral and contact toxicity study on bees has been performed with bentazone according to recent guideline.
In addition, a toxicity study on acute oral and contact toxicity on bees was performed with bentazone in accordance with UK working document 7/3 (1986) and was assessed in the EU review of bentazone.

Acute oral toxicity	bentazone: LD_{50} (24h) > 200.0 µg a.s./bee
Acute contact toxicity	bentazone: LD_{50} (24 h) > 200.0 µg a.s./bee
Acute oral toxicity	bentazone: LD_{50} (48 h) > 159.16 µg a.s./bee
Acute contact toxicity	bentazone: LD_{50} (48 h) > 150.0 µg a.s./bee

Effects on other arthropod species (OECD data point numbers Annex IIA, 8.8)

Studies on non-target terrestrial arthropods are generally not performed with the pure active ingredient bentazone, but with the representative formulation BAS 351 32 H. For further details please refer to Document M-III, Chapter 10.5.
Additional studies with the formulation BAS 351 45 H were submitted with the Monograph (Vol. 3, Annex B.8, September 1996) and are stated in the EC Review Report (7585/VI/97-final, November 2000).

Effects on earthworms (OECD data point number Annex IIA, 8.9)

Acute (14-day) earthworm studies were carried out with the active substance **bentazone** and **bentazone-Na** and the relevant metabolite **N-methyl-bentazone (M351H009)**. For the metabolite **N-methyl-bentazone**, a sublethal earthworm study was carried out in addition.

Acute toxicity on earthworms [mg/kg dry soil]	bentazone: LC_{50} > 1000 bentazone-Na: LC_{50} > 1000 N-methyl-bentazone: LC_{50} > 1000
Sublethal effects on earthworms [mg/kg dry soil]	N-methyl-bentazone: LC_{50} > 14.0

Effects on soil micro-organisms (OECD data point number Annex IIA, 8.10)

Studies on soil micro-organisms (effects of on carbon and nitrogen transformation) were carried out with the active substance **bentazone** and **bentazone-Na** and the relevant metabolite **N-methyl-bentazone (M351H009)**.

Nitrogen and carbon transformation	**bentazone:** No adverse long-term effects (deviations compared to the control were < 25% at the end of the studies (56 days) and thus were below the trigger of the OECD guideline 216) on nitrogen transformation were observed up to and including 5.76 mg a.s./kg dry soil. No adverse short-term or long-term effects (deviations compared to the control were < 25% at the end of the studies (28 days) and thus were below the trigger of the OECD guideline 217) on carbon transformation were observed up to and including 19.2 mg a.s./kg dry soil. **bentazone-Na:** No adverse long-term effects (deviations compared to the control were < 25% at the end of the studies (42 days) and thus were below the trigger of the OECD guideline 216) on nitrogen transformation were observed up to and including 6.27 mg/kg dry soil. **N-methyl-bentazone:** No adverse short-term or long-term effects (deviations compared to the control were < 25% at the end of the studies (28 days) and thus were below the trigger of the OECD guidelines 216 and 217) on nitrogen and carbon transformation were observed up to and including 2.0 mg/kg dry soil.

Effects on non-target terrestrial plants (OECD data point number Annex IIA, 8.12)

Studies on non-target plants have been performed with BAS 351 32 H and are reported in Document M-III, Chapter 10.8.

Effects on biological methods of sewage treatment (OECD data point numbers Annex IIA, 8.15)

Bentazone did not have significant inhibitory effects on the respiration rate of activated sludge collected from a laboratory wastewater plant (Doc ID 2004/1025743). The EC_{20} and EC_{50} values of bentazone in the activated sludge respiration inhibition test are both > 1000 mg a.s./L, respectively.

Disturbances in the bio-degradation process of activated sludge are not to be expected if the test item is correctly introduced into adapted wastewater treatment plants at low concentrations.

Respiration inhibition test (activated sludge)	**bentazone:** EC_{20} / EC_{50} > 1000 mg a.s./L

Other/special studies (OECD data point number Annex IIA, 8.16)

No other or special studies than those detailed under Document M-II, Chapters 8.1 to 8.15 were performed or are deemed to be necessary.